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THE FOREIGN BODY RESPONSE TO CENTRAL  
NERVOUS SYSTEM IMPLANTS

by

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A dissertation submitted to the faculty of  
The University of Utah  
in partial fulfillment of the requirements for the degree of

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# The University of Utah Graduate School

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## ABSTRACT

Implanted biomedical devices play an important role in the treatment of central nervous system diseases and disorders, but are also subject to a foreign body response which has the potential to affect device function. For example, the recordings obtained from the cerebral cortex using penetrating microelectrode arrays have been shown to be sufficient in limited clinical trials to control computers or external devices in the field of neuroprosthetics, but such recordings are largely inconsistent in chronic applications. Following implantation, a local area of inflammation develops surrounding the implant, which consists of activated microglia/macrophages, astrocyte hypertrophy, and neuronal loss, and which appears to last for the lifetime of the implant. The focus of this dissertation was to investigate the local tissue response in rat cerebral cortex adjacent to microwires, silicon microelectrodes with different surface chemistries, and microelectrode designs with decreased surface area available for inflammatory cell attachment. In addition, the effect of the implant-associated foreign body response to hippocampal neurogenesis was elucidated. We observed that the chronic tissue response to implanted microwires and silicon microelectrodes is similar to other devices implanted in the brain, such as the deep brain stimulator and cerebrospinal shunt, and consisted of chronic inflammation and neuronal loss within the first 50  $\mu\text{m}$  from the electrode-tissue interface. Local neuronal loss did not progress from 2 to 12 weeks, and astrocytes did not form a syncytium. Surface chemistry appeared to play only a minor role in the tissue

reaction, suggesting that other design variables, such as porosity, stiffness, or implant size/surface area may play a larger role. We also found that the implantation of penetrating brain implants was sufficient to decrease hippocampal neurogenesis, regardless of implant location, in all cases but lattice implants with reduced surface area/stiffness. This suggests that the tissue response may be passively antagonized by design variables built into future CNS devices.

To my wife, Anna.

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## LIST OF ABBREVIATIONS

| <u>Defining Term</u>                                | <u>Abbreviation</u> |
|-----------------------------------------------------|---------------------|
| 4', 6-diamidino-2-phenylindole dihydrochloride..... | DAPI                |
| Analysis of variance.....                           | ANOVA               |
| Blood-brain barrier.....                            | BBB                 |
| Bromodeoxyuridine .....                             | BrdU                |
| Central nervous system.....                         | CNS                 |
| Cerebrospinal fluid.....                            | CSF                 |
| Deep brain stimulator.....                          | DBS                 |
| Deionized .....                                     | DI                  |
| Doublecortin .....                                  | DcX                 |
| Dentate gyrus .....                                 | DG                  |
| Deoxyribonucleic acid .....                         | DNA                 |
| Ethylene oxide .....                                | EtO                 |
| Experimental autoimmune encephalitis.....           | EAE                 |
| Foreign body response .....                         | FBR                 |
| Fourier transform infrared spectroscopy.....        | FTIR                |
| Glial fibrillary acidic protein .....               | GFAP                |
| Institutional animal care and use committee.....    | IACUC               |
| Interleukin-6.....                                  | IL-6                |

|                                               |               |
|-----------------------------------------------|---------------|
| Intelligence quotient.....                    | IQ            |
| Lipopolysaccharide .....                      | LPS           |
| Myelin associated protein-2 .....             | MAP-2         |
| Monocyte chemoattractant protein .....        | MCP           |
| Myelin basic protein .....                    | MBP           |
| Myelin-oligodendrocyte specific protein ..... | MOSP          |
| Neuronal nuclei .....                         | NeuN          |
| Neurofilament-160 .....                       | NF-160        |
| Neurofilament-200 .....                       | NF-200        |
| Phosphate-buffered saline .....               | PBS           |
| 3,4-ethylenedioxythiophene.....               | PEDOT         |
| Scanning electron microscopy .....            | SEM           |
| Silicon microelectrode array .....            | SiMEA         |
| Signal-to-noise ratio.....                    | SNR           |
| Stainless steel .....                         | SS            |
| Traumatic brain injury .....                  | TBI           |
| Tumor necrosis factor-alpha .....             | TNF- $\alpha$ |
| Ultraviolet .....                             | UV            |

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## CHAPTER 1

### INTRODUCTION

#### CNS Injury and Disease

Disease and injury to the central nervous system (CNS), consisting of the brain and spinal cord, can be extremely debilitating to patients and costly to caregivers. Such diseases and injuries affect over 50 million Americans at an annual cost of approximately \$400 billion. One such disease is Alzheimer's disease, which is a progressive neurodegenerative disorder that affects approximately 5 million Americans [1] at an annual cost estimated to be \$100 billion [2], and consists of cognitive and memory deterioration and impairment of behavior [3]. Another common progressive neurodegenerative disorder is Parkinson's disease, which consists of a loss of dopaminergic neurons in the pars compacta region of the substantia nigra. Clinical manifestations consist of rigidity, rapid repetitive tremor, and bradykinesia, affecting motor skills as well as speech [4]. Parkinson's disease is estimated to affect approximately 350,000 Americans [5] at an annual cost of \$24 billion [6]. Another is Multiple sclerosis, an autoimmune disorder in which white matter of the CNS is destroyed [7], and is estimated to affect in excess of 350,000 Americans [8] at an annual cost of over \$7 billion [9]. Hydrocephalus is either a developmental or acquired disorder in which abnormal amounts of cerebrospinal fluid (CSF) accumulate in the brain, causing

increased intracranial pressure and herniation often requiring surgery. Hydrocephalus affects approximately 700,000 Americans [10] at an annual cost exceeding \$1 billion [11]. Traumatic brain injury (TBI) with multiple levels of severity, often resulting in cognitive and physical deficits, affects approximately 5 million Americans at an annual cost estimated to be over \$60 billion [12]. Spinal cord injury (SCI) affects another 250,000 Americans at an annual cost of \$4 billion [13].

### Therapeutic Biomaterials for CNS Injury and Disease

With such staggering numbers of patients affected and the high cost to society, a number of therapies and medical devices are currently approved or under investigation to manage clinical symptoms of CNS diseases and disorders, the vast majority of which are designed as chronic applications. Many such therapies and medical devices are biomaterial-based [14, 15], including metallic, polymeric, ceramic, and composite materials. One example is the hydrocephalic shunt, which is used to drain excess CSF in patients with congenital or acquired hydrocephalus and spina bifida. Treatment consists in the placement of a silicone tube in the affected ventricle, serving as a conduit for CSF to exit the brain, often into the peritoneal cavity [16]. CSF shunt placement remains the standard of care for patients with hydrocephalus, and indeed is one of the most common neurosurgical procedures performed [11].

Stimulating electrodes are another category of devices used to treat CNS disorders. The cochlear implant [17], which stimulates the inner ear tonotopically to treat deafness, has helped thousands of profoundly deaf patients to hear. The deep brain stimulator [18, 19] is a bilateral array of Pt-Ir stimulating electrodes which are implanted chronically in the thalamus or basal ganglia to treat the symptoms associated with



Parkinson's disease [20], and depression [21]. In addition, the deep brain stimulator is currently being investigated for use in treating obsessive-compulsive disorder [22] and epilepsy [23], as well as a host of other neurological disorders and neuropathic pain.

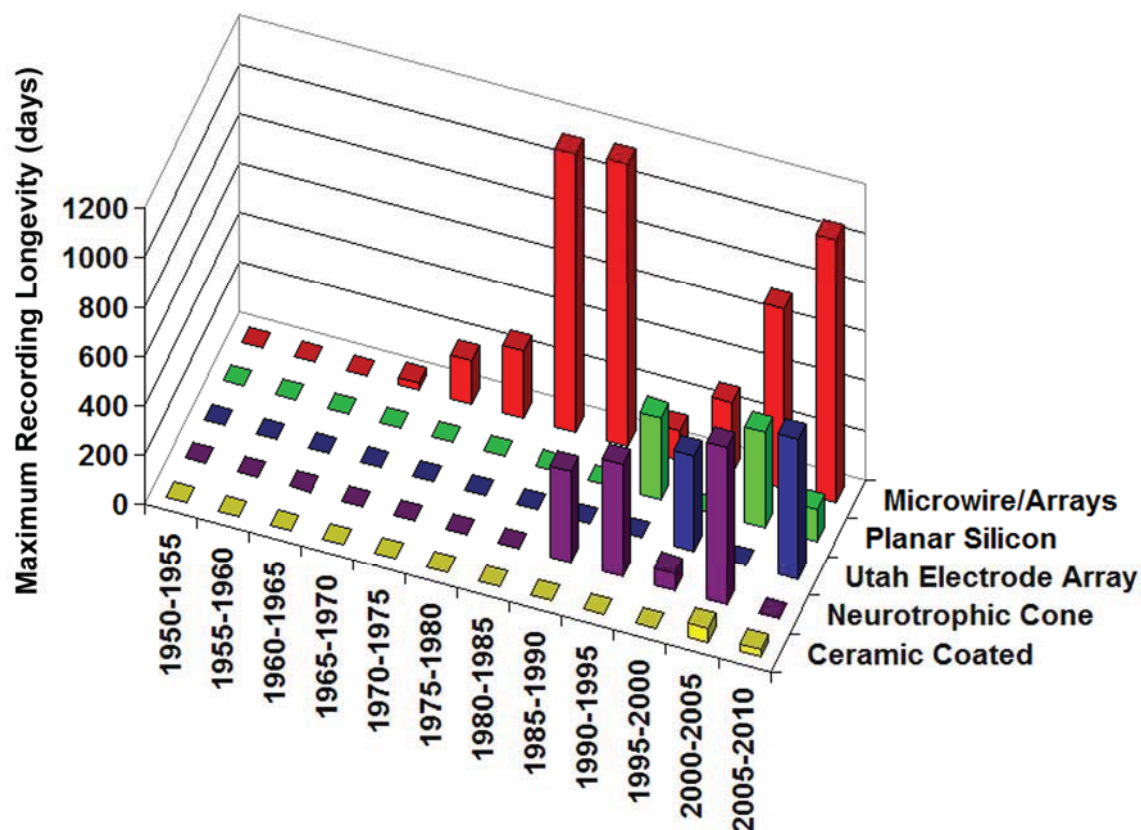
Other CNS electrode applications, broadly termed neuroprosthetic applications or brain-computer interfaces, have the goal of connecting a patient's brain to a computer or assistive device to restore function, and have the potential to serve a much larger patient population, treating patients with motor impairments resulting from spinal cord injury, stroke, limb loss, or neuromuscular disorders [24-26]. These devices consist of a recording electrode array, implanted into the patient's cerebral cortex to extract volitional signals, extraction algorithms to decode them, and mechanical actuators or computers to provide outputs [27]. The electrode arrays generally consist of sets of microwires [28-30], or silicon microelectrode arrays (SiMEAs) [31, 32]. Recent human clinical trials utilizing a silicon microelectrode array demonstrated the ability of a tetraplegic patient to control a computer cursor, prosthetic hand, and a robotic arm using signals extracted from the patient's brain, utilizing the patient's own volitional thoughts [33].

### Limitations of Current Technologies

CSF shunts are silastic tubes that divert excess CSF from the ventricles to other body cavities. As described previously, they have had much success in treating hydrocephalus, but nonetheless, shunt complication rates remain very high [34]. Many shunt complications are due to bacterial or viral infection, which necessitates the surgical removal of the infected shunt [35, 36]. In addition, obstruction of the catheter lumen is also very common, which is believed to be related to the biological reaction the brain mounts against the implant [37], also requiring surgical revision.

DBS electrodes have widespread use in treating the tremor associated with Parkinson's disease. However, there are also problems associated with this device. Infection rates vary from 1 – 15%, with infections occurring sometimes years following device implantation [38-41]. Sudden failure of the device or intermittent function has also been described in multiple clinical reports [42-44]. Failure is often attributed to mechanical effects, but may be related to the biological response, which will be described in the next section.

The goal of chronic recording in neuroprosthetic applications is to obtain as many single units with high signal-to-noise ratio (SNR) as possible over the lifetime of the implant [45]. In order to obtain such recordings in the cerebral cortex, the implanted electrode must be in close association with viable neurons, since the maximum recordable distance between signal origin and recording electrode has been shown to be on the order of 50 – 100  $\mu\text{m}$  [46-48]. With such spatial limitations imposed on recording, the stability of the electrode-tissue interface is of great concern especially in chronic applications. Many studies in the literature, in a variety of species and with multiple types of electrode arrays, have shown chronic cortical recording to be inconsistent [30, 49-52], summarized in Figure 1-1 and Table 1.1. What is clear from this group of studies is that microwire arrays often outperform other electrode designs in terms of maximum recording longevity, and that most designs are appropriate for clinical applications. However, what is not clear is the correlation between the tissue response and device function. As early as 1974, Burns, et al. showed a progressive decline in unit recordings in cat cerebral cortex after implantation, with only 8% of electrodes functioning after 5 months [49]. A quarter of a century later, Liu et al. reported that implanted electrodes



**Figure 1-1.** Maximal recording longevity as a function of electrode type. Survey of the peer reviewed literature showing the maximum reported recording lifetimes reported by half decade as a function of electrode type. Note the early and unmatched success of microwires and microwire arrays in terms of maximal recording longevity with several reports as early as the 1980s showing recording exceeding 3 years. In addition, a very recent report has shown chronic recording capability in a nonhuman primate model exceeding 7 years with a simple microwire array [53] (not shown in figure).

**Table 1.1 Electrode Recording Duration Summary**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>   | <b>Functional<br/>Recording</b> | <b>Electrode Type</b>                      | <b>Scientific Contribution</b>                                                                                            |
|------------------------------|---------------|--------------------|---------------------------------|--------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| 1967<br>[54]                 | Naka          | Cat                | 30 days                         | Araldite insulated<br>Tungsten wire        | Animal's emotional state can alter recording<br>ability                                                                   |
| 1972<br>[55]                 | Chorover      | Rat/<br>7 total    | 90 days                         | Teflon insulated<br>Pt-Ir (7 microwires)   | First report of recordings of spontaneous and<br>stimulus-evoked responses using microwire<br>recording array             |
| 1974<br>[49]                 | Burns         | Cat/<br>9 total    | 175 days                        | Pt with Diamel<br>varnish (7 microwires)   | Potential of tissue reaction adversely<br>affecting recording ability, and of electrodes<br>migrating over time described |
| 1974<br>[56]                 | Suzuki        | Cat/               | 90 days                         | Stainless steel<br>(8 microwires)          | Recordings can be made from freely moving<br>animals using appropriate hardware                                           |
| 1976<br>[50]                 | Schmidt       | Monkey/<br>1 total | 223 days                        | Parylene-insulated<br>Ir (12 microwires)   | Electrode implant results in a foreign body<br>response; precipitous drop in impedance<br>noted between 20-30 days        |
| 1977<br>[57]                 | Loeb          | Monkey/<br>2 total | 275 days                        | Parylene-insulated<br>Ir (12 microwires)   | Correlation of impedance decline and<br>reduction in recording ability                                                    |
| 1978<br>[58]                 | Durelli       | Cat/3              | 23 days                         | Parylene-insulated<br>Ir (12 microwires)   | Recordings unstable immediately after<br>implant; instability increases over time                                         |
| 1978<br>[59]                 | Palmer        | Cat/3              | 42 days                         | Teflon-insulated<br>Pt-Ir (4-6 microwires) | Analysis of recording stability, impedance,<br>and SNR over time                                                          |

**Table 1.1 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>    | <b>Functional<br/>Recording</b> | <b>Electrode Type</b>                       | <b>Scientific Contribution</b>                                                                                               |
|------------------------------|---------------|---------------------|---------------------------------|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|
| 1980<br>[60]                 | Schmidt       | Monkey/<br>1        | 1125 days                       | Parylene-insulated<br>Ir (12 microwires)    | Follow-up of 1976 report, indicating the possibility of recording up to 3 years                                              |
| 1981<br>[61]                 | Fontani       | Rabbit/<br>22 total | 30 days                         | Nichrome (6<br>microwires)                  | Techniques to precisely localize the position of Nichrome wires ex vivo                                                      |
| 1984<br>[62]                 | Legendy       | Cat/<br>9 total     | 25 days                         | Parylene-insulated<br>Ir (2-5 microwires)   | Similar recording setup as the Schmidt group, but with recordings for less than a month shows the variability between groups |
| 1988<br>[51]                 | Schmidt       | Monkey/<br>6 total  | 1144 days                       | Parylene-insulated<br>Ir (12 microwires)    | Correlated decreased impedance with the cracking of Parylene insulative layer                                                |
| 1988<br>[63]                 | Mioche        | Cat/<br>27 total    | 28 days                         | Teflon-insulated<br>Pt-Ir (1-19 microwires) | First report of stimuli-evoked recordings in visual cortex (rationale for visual prosthesis)                                 |
| 1989<br>[64]                 | Kennedy       | Rat/<br>30 total    | 365 days                        | Neurotrophic cone<br>electrode              | First report of recoding ability and tissue response of the neurotrophic cone electrode                                      |
| 1990<br>[65]                 | Palmer        | Cat/<br>2 total     | 61 days                         | Teflon-insulated<br>Pt-Ir (10 microwires)   | Electrode migration hypothesized to be the major factor underlying recording instability                                     |
| 1991<br>[66]                 | Merzhanova    | Cat/<br>6 total     | 120 days                        | Nichrome (4-7<br>microwires)                | Novel spike sorting analysis system for differentiating cells recorded simultaneously                                        |
| 1992<br>[67]                 | Kennedy       | Monkey/<br>2 total  | 455 days                        | Neurotrophic cone<br>electrode              | Full report of the tissue response within and adjacent to the neurotrophic cone electrode                                    |

**Table 1.1 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>           | <b>Functional<br/>Recording</b> | <b>Electrode Type</b>                               | <b>Scientific Contribution</b>                                                            |
|------------------------------|---------------|----------------------------|---------------------------------|-----------------------------------------------------|-------------------------------------------------------------------------------------------|
| 1993<br>[68]                 | Carter        | Rat/<br>42 total           | 365 days                        | Planar silicon<br>Michigan array                    | First description of recording using silicon-based microelectrode arrays                  |
| 1994<br>[69]                 | Hetke         | Guinea<br>Pig/<br>10 total | 330 days                        | Planar silicon<br>Michigan array                    | First report of the performance of the full planar Michigan microelectrode array          |
| 1997<br>[70]                 | Westby        | Rat/<br>42 total           | 270 days                        | Teflon-insulated<br>Pt-Ir (6 microwires)            | Report of a floating microwire array system used to record in rats for a period of months |
| 1998<br>[71]                 | Rousche       | Cat/<br>10 total           | 390 days                        | Utah electrode array                                | Report of impedance decreasing over the life of a silicon-based recording array           |
| 1998<br>[72]                 | Kennedy       | Human/<br>1 total          | 69 days<br>(patient death)      | Neurotrophic cone<br>electrode                      | First report of a recording-based clinical neuroprosthetic application                    |
| 1999<br>[52]                 | Liu           | Cat/<br>11 total           | 292 days                        | Epoxylite-insulated<br>Ir (7 microwires)            | No correlation found between recording stability and histologically stained neurons       |
| 1999<br>[28]                 | Williams      | Guinea<br>Pig/8 total      | 283 days                        | Tungsten (33<br>microwires)                         | Methods paper for assembling a novel microwire array and recording                        |
| 1999<br>[73]                 | Venkatachal   | Rat/<br>5 total            | 60 days                         | Parylene-insulated<br>Tungsten (6 microwires)       | First report of cell-specific immunomarkers used to describe the tissue response          |
| 2000<br>[74]                 | Wessberg      | Monkey/<br>2 total         | 730 days                        | Teflon-insulated stainless<br>steel (96 microwires) | Cortical activity has sufficient information to control 1-D movement of a robotic arm     |

**Table 1.1 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>      | <b>Functional<br/>Recording</b> | <b>Electrode Type</b>                               | <b>Scientific Contribution</b>                                                                                 |
|------------------------------|---------------|-----------------------|---------------------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| 2000<br>[75]                 | Kennedy       | Human/<br>3 total     | 426 days                        | Neurotrophic cone<br>electrode                      | Report of the neurotrophic cone electrode<br>used to control a computer cursor                                 |
| 2000<br>[76]                 | Porada        | Monkey/<br>1 total    | 700 days                        | Polyimide insulated<br>Ni-Cr-Al (64 microwires)     | Electrodes that record spikes, become<br>quiescent, then record again described                                |
| 2002<br>[77]                 | Taylor        | Monkey/<br>2 total    | 730 days                        | Teflon-insulated stainless<br>steel (64 microwires) | Recordings sufficient to control the 3-D<br>movements of a robotic arm for 3 years                             |
| 2003<br>[29]                 | Nicolelis     | Monkey/<br>3 total    | 550 days                        | Stainless steel (16-32<br>microwires)               | Longer recording stability associated with<br>slow array insertion                                             |
| 2003<br>[78]                 | Cui           | Guinea<br>Pig/6 total | 14 days                         | Polypyrrole-coated<br>Michigan array                | Recording quality does not correlate with<br>impedance of the Michigan array                                   |
| 2003<br>[79]                 | Kipke         | Rat/<br>6 total       | 382 days                        | Planar silicon<br>Michigan array                    | Feasibility of using the Michigan array in<br>chronic experiments explored                                     |
| 2004<br>[80]                 | Vetter        | Rat/<br>10 total      | 127 days                        | Planar silicon<br>Michigan array                    | Recordings over one month showed<br>decreases in SNR and impedance                                             |
| 2004<br>[81]                 | Musallam      | Monkey/<br>3 total    | 60 days                         | Parylene-insulated<br>Tungsten (96 microwires)      | High level motor signals decoded and used<br>to position a computer cursor                                     |
| 2004<br>[82]                 | Kennedy       | Human/<br>2 total     | 636 days                        | Neurotrophic cone<br>electrode                      | Volitional signals recorded and transmitted<br>wirelessly to control a computer speller and<br>a virtual digit |

**Table 1.1 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>   | <b>Functional<br/>Recording</b> | <b>Electrode Type</b>                        | <b>Scientific Contribution</b>                                                                                                       |
|------------------------------|---------------|--------------------|---------------------------------|----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| 2005<br>[83]                 | Rennaker      | Rat/<br>21 total   | 42 days                         | Tungsten (14<br>microwires)                  | Insertion method affects chronic recording<br>capability with mechanically inserted arrays<br>outperforming manually inserted arrays |
| 2005<br>[84]                 | Johnson       | Rat/<br>6 total    | 131 days                        | Planar silicon<br>Michigan array             | Repeated voltage biasing can improve SNR                                                                                             |
| 2005<br>[85]                 | Suner         | Monkey/<br>3 total | 569 days                        | Parylene-insulated<br>Utah electrode array   | Reliability study of the Utah electrode array<br>prior to human clinical trials                                                      |
| 2006<br>[86]                 | Ludwig        | Rat/<br>8 total    | 42 days                         | Planar silicon<br>Michigan array             | PEDOT coating significantly improves<br>recording ability of Michigan array                                                          |
| 2006<br>[87]                 | McCreery      | Cat/<br>11 total   | 415 days                        | Parylene-insulated<br>Ir (16 microwires)     | Performance of a combination recording/<br>stimulating array described                                                               |
| 2006<br>[33]                 | Hochberg      | Human/<br>2 total  | 330 days                        | Utah electrode array                         | Volitional signals from a SCI patient able<br>to control a computer cursor and robotic arm                                           |
| 2006<br>[30]                 | Liu           | Cat/<br>25 total   | 1061 days                       | Parylene-insulated<br>Ir (16 microwires)     | Detailed study on recording longevity with<br>large number of animals and clinically<br>relevant timepoints                          |
| 2007<br>[88]                 | Musallam      | Rat/<br>4 total    | 90 days                         | Parylene-insulated<br>Pt-Ir (18 microwires)  | Performance of a floating microwire array<br>described                                                                               |
| 2007<br>[89]                 | Jackson       | Monkey/<br>2 total | 326 days                        | Teflon-insulated<br>Tungsten (12 microwires) | Performance of a movable microwire array<br>along with endpoint histology is described                                               |



**Table 1.1 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>   | <b>Functional<br/>Recording</b> | <b>Electrode Type</b>                           | <b>Scientific Contribution</b>                                              |
|------------------------------|---------------|--------------------|---------------------------------|-------------------------------------------------|-----------------------------------------------------------------------------|
| 2008<br>[90]                 | Yamamoto      | Rat/<br>21 total   | 150 days                        | Polyimide-insulated<br>Nichrome (tetraode)      | Motorized microdrive system for controlling<br>electrode position described |
| 2008<br>[91]                 | Eliades       | Monkey/<br>2 total | 447 days                        | Epoxylite-insulated<br>Tungsten (16 microwires) | System for recording from freely moving,<br>caged monkeys is presented      |
| 2010<br>[53]                 | Kruger        | Monkey/<br>1 total | 2729 days                       | Polyimide-insulated<br>Nichrome (64 microwires) | Clinical timescale recordings possible in<br>nonhuman primates              |

have low recording stability during tissue remodeling, and thereafter experience a continual decrease in recording ability over the ensuing months [52]. Hypotheses regarding chronic recording instability have focused on the electrode itself, including loss of the insulating layer or movement and micromotion [50, 92, 93], or on the tissue reaction that the brain mounts against implants [94, 95]. Available evidence suggests that the brain tissue response is the greatest challenge to obtaining consistent recordings, and since the inflammatory tissue reaction may extend several hundred micrometers, that recording ability may be intimately tied to the tissue reaction [45]. However, after some 65 years of studying the brain tissue reaction to implanted electrodes [96], the precise mechanisms by which the foreign body response limits chronic recordings remain unclear. Therefore, an understanding of the factors that affect the brain tissue response to implanted electrodes is a critical barrier to progress in the field of neuroprosthetics.

#### Placement of Biomaterial Devices in the *In Vivo* Environment

The placement of any therapeutic biomaterial-based device in the *in vivo* environment requires injection, insertion or surgical implantation, all of which damages the target tissues [97]. Following tissue damage, a host of processes are set in motion to affect wound closure and the creation of a new tissue surrounding the implant, in a continuum of processes called wound healing [98]. Initial damage is often to the local vasculature during implantation, which initiates the coagulation cascade to form a provisional matrix in the area of damage [99]. The complement system can also become activated, leading to cell death through formation of the membrane attack complex, as well as the recruitment of neutrophils and the stimulation of the inflammatory response [100]. Inflammation ensues with the migration of neutrophils to the injury site, with roles

in tissue cleanup, phagocytosis of complement protein-opsinized cells, and recruitment of additional inflammatory cells, via binding to adsorbed proteins [101]. In the presence of persistent inflammatory stimuli, such as a biomaterial implant, eventually macrophages become the predominant cell type at the biomaterial-tissue interface, resulting in chronic inflammation [102]. Macrophages bind to the surface of implanted materials, and secrete proinflammatory cytokines which causes the inflammatory reaction to persist [103]. Eventually, macrophages may fuse into foreign body giant cells during the foreign body reaction which may persist at the biomaterial-tissue interface for the lifetime of the implant [104]. A similar wound healing reaction has been described following electrode implantation in the brain, with seminal contributions from the literature summarized in Table 1.2 and Figure 1-2.

The brain tissue reaction to implanted electrodes is similar to the foreign body response (FBR) observed in other tissues [105, 106], and involves activated microglia/macrophages, astrocytes, neurons, oligodendrocytes, the surrounding cerebral vasculature, and precursor cells. Historically, the distinguishing features of the brain tissue response to cortically implanted electrodes were initially qualitatively described using histological markers in tissue sections surrounding implants which were thin-sectioned. Due to the tight regulation of blood constituents reaching brain tissue by the blood-brain barrier (BBB), resident microglial cells of the brain represent the local immune system, and dynamically sample the extracellular space [107]. Following injury, such as electrode implantation, the BBB is compromised due to vascular damage, and blood-borne macrophages enter the brain and become indistinguishable from resident microglia [108, 109]. Microglia/macrophages then transform morphologically and

**Table 1.2 Brain Tissue Response to Electrodes**

| <b>Year</b>   | <b>Author</b> | <b>Species/N</b>      | <b>Timepoint</b>   | <b>Electrode Type</b>              | <b>Contribution</b>                                                                                                                                          |
|---------------|---------------|-----------------------|--------------------|------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>[Ref.]</b> |               |                       |                    |                                    |                                                                                                                                                              |
| 1943<br>[96]  | Baggenstoss   | Human/<br>(70 total)  | 1 day – 7 years    | SS Needle                          | Degeneration of cells close to implant site<br>Swollen microglia surround wound site at each timepoint<br>Hypertrophic astrocytes encapsulate implant        |
| 1955<br>[110] | Dodge         | Human/<br>(1 total)   | 19 months          | Cu Wire<br>Formvar insulated       | White matter and tissue loss surround electrode                                                                                                              |
| 1957<br>[111] | Fischer       | Cat/7<br>(28 total)   | 1 – 4 weeks        | Ag-AgCl Wire<br>Cu wire<br>SS Wire | Zone of swollen microglia surround wires<br>Leukocyte trafficking from vessels<br>Reaction is progressive over time<br>Material composition affects response |
| 1957<br>[105] | Collias       | Cat/2<br>(16 total)   | 24 days – 6 months | SS Wire<br>Arrays                  | Capsule formation around electrodes<br>Reaction similar in different brain compartments                                                                      |
| 1963<br>[112] | Levine        | Rat/15<br>(600 total) | 1 day – 1 week     | Graphite                           | Myelin/Oligodendrocyte loss near implant site with axon preservation<br>Acute BBB disruption<br>Response similar to neurodegenerative disease state          |
| 1969<br>[113] | Wetzel        | Cat<br>(6 total)      | 1 hour – 11 weeks  | Pt or SS wire                      | Tissue response includes neuronal loss, and may affect stimulation ability                                                                                   |
| 1970<br>[114] | Dymond        | Cat/NA                | 2 months           | Pt Wire<br>Pt-Alloys               | Comparison of tissue response of indwelling electrode to stab wounds                                                                                         |

**Table 1.2 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>      | <b>Timepoint</b> | <b>Electrode Type</b>                                                                       | <b>Contribution</b>                                                                                              |
|------------------------------|---------------|-----------------------|------------------|---------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| 1970                         | Dymond        | (continued)           |                  | Au-Ni-Cr Wire<br>Au-Pd-Rh Wire<br>Vitalium Wire<br>SS Wire<br>Ag Wire<br>Re Wire<br>Au Wire | Classified potential materials for electrodes with regards to toxicity potential                                 |
| 1974<br>[49]                 | Burns         | Cat/<br>(9 total)     | 42 – 179 days    | Pt Wire                                                                                     | Suggest that tissue reaction may adversely affect recording electrode function                                   |
| 1975<br>[115]                | Robinson      | Rat/2<br>(16 total)   | 1 – 60 days      | Pt Wire                                                                                     | Cells adjacent to implanted electrode undergo structural and metabolic changes                                   |
| 1976<br>[116]                | Boast         | Mouse/6<br>(11 total) | 10 days          | Teflon coated SS                                                                            | Electrode implantation likely causes persistent damage to the BBB                                                |
| 1976<br>[50]                 | Schmidt       | Monkey/<br>(1 total)  | 223 days         | Parylene-C coated Ir                                                                        | Electrode implantation causes a FBR                                                                              |
| 1976<br>[94]                 | Schultz       | Cat/12                | 26 – 328 days    | Epoxylite coated SS                                                                         | Neuronal loss adjacent to zone of gliosis<br>Foreign body giant cells at interface<br>Meningeal cells in capsule |
| 1976<br>[106]                | Stensaas      | Rabbit/22<br>Rat/24   | 4 – 723 days     | Araldite needles                                                                            | Similar reaction in different species                                                                            |

**Table 1.2 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>      | <b>Timepoint</b> | <b>Electrode Type</b>                                                                                                | <b>Contribution</b>                                                                                                                                         |
|------------------------------|---------------|-----------------------|------------------|----------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1978<br>[117]                | Levine        | Rat/3<br>(90 total)   | 1 day – 4 weeks  | Al wires<br>Be wires<br>Cr wires<br>Fe wires<br>Bi wires<br>Cd wires<br>Co wires<br>Cu wires<br>Ni wires<br>Zn wires | Tissue reaction indistinguishable from EAE, further suggesting a similarity between the tissue response to implanted materials and neurodegenerative states |
| 1978<br>[118]                | Dodson        | Baboon/1<br>(4 total) | 8 days           | Pt Wire                                                                                                              | Tissue response not symmetric around wire<br>Tissue response divided into developmental states                                                              |
| 1978<br>[119]                | Stensaas      | Rabbit/>27            | 50 – 723 days    | Al wire<br>Au wire<br>Pt wire<br>Silicon<br>Ag wire<br>Fe wire<br>Cu wire<br>Co wire                                 | First attempt to quantify tissue reactivity<br>A variety of materials elicit minimal tissue response                                                        |
| 1986<br>[120]                | Agnew         | Cat/<br>(64 total)    | 24 – 33 days     | Pt-Ir<br>Ir                                                                                                          | Suggest that neuronal degeneration is secondary to necrosis at tissue interface                                                                             |

**Table 1.2 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>     | <b>Timepoint</b>   | <b>Electrode Type</b>   | <b>Contribution</b>                                                                                                                                         |
|------------------------------|---------------|----------------------|--------------------|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1988<br>[51]                 | Schmidt       | Monkey<br>(4 total)  | 3 years            | Parylene-C<br>coated Ir | Increase in recording instability not<br>correlated with breakdown of the insulative<br>layer                                                               |
| 1992<br>[93]                 | Edell         | Rabbit/2<br>12 total | 6 months           | Silicon                 | Quantified neuronal density as a function of<br>distance from the electrode<br>Neuronal loss mainly within 50 $\mu\text{m}$ from the<br>electrode interface |
| 1993<br>[121]                | Schmidt       | Cat/12<br>(27 total) | 24 hrs – 6 months  | Silicon<br>(UEA)        | Activated macrophages at the interface<br>likely persist for the lifetime of the implant                                                                    |
| 1994<br>[122]                | Caparros      | Human/<br>(1 total)  | 43 months          | Pt-Ir<br>DBS            | Neuronal lesion associated with FBR<br>First report of tissue reaction with clinical<br>DBS                                                                 |
| 1999<br>[52]                 | Liu           | Cat<br>(11 total)    | 242 days           | Epoxyite<br>coated Ir   | No correlation found between recording<br>stability and immunomarkers for neurons                                                                           |
| 1999<br>[123]                | Turner        | Rat/3<br>(16 total)  | 2 – 12 weeks       | Silicon                 | Described unidentified cellular attachment<br>to explanted silicon electrodes                                                                               |
| 2000<br>[124]                | Haberler      | Human<br>(8 total)   | 2 days – 70 months | Pt-Ir<br>DBS            | Tissue reaction to DBS electrodes includes<br>astrocytosis, microglia at the interface, and<br>a fibrous capsule                                            |
| 2003<br>[125]                | Shain         | Rat                  | 1 – 6 weeks        | Silicon                 | Peripheral dexamethasone injections may<br>attenuate the glial response                                                                                     |

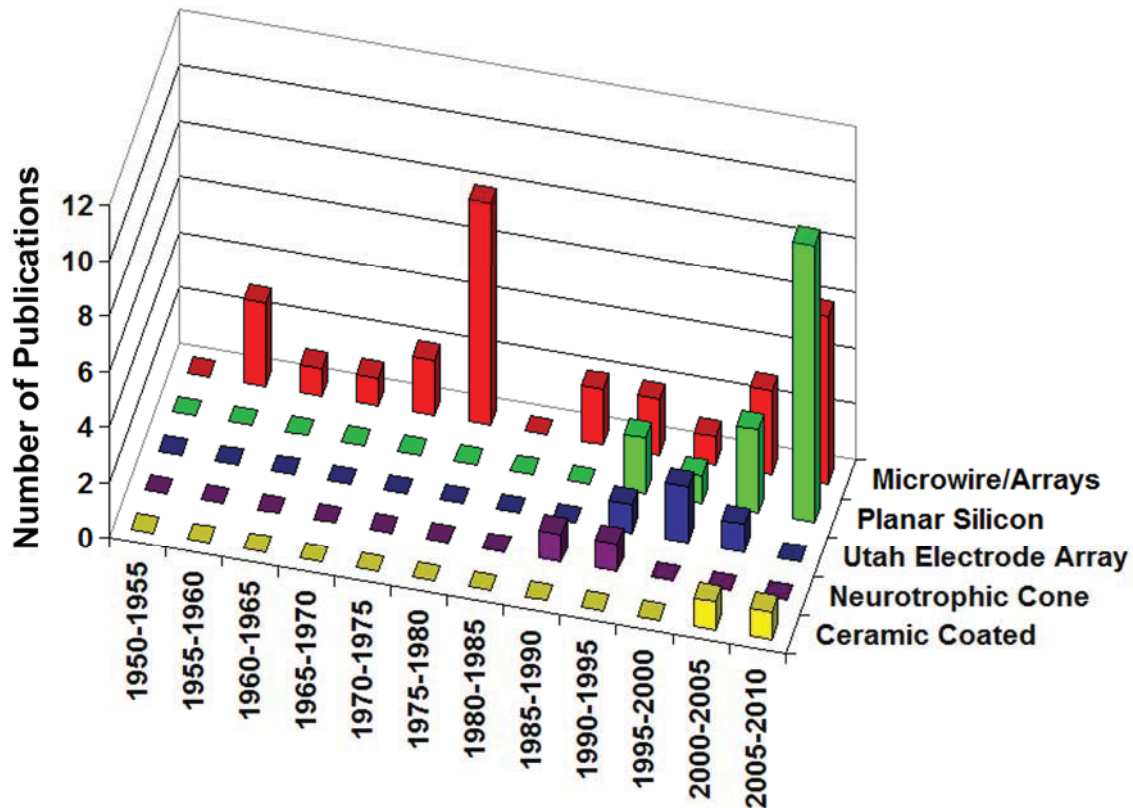
**Table 1.2 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>    | <b>Timepoint</b> | <b>Electrode Type</b> | <b>Contribution</b>                                                                                                                                                                                                                     |
|------------------------------|---------------|---------------------|------------------|-----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2003<br>[126]                | Szarowski     | Rat/2               | 1 day – 12 weeks | Silicon               | Cells adherent to explanted electrodes are activated macrophages/microglia                                                                                                                                                              |
| 2004<br>[127]                | Kim           | Rat/4<br>(12 total) | 4 weeks          | HFM                   | Quantitative image analysis to describe tissue response<br>Compared fixation methods on the ensuing tissue response                                                                                                                     |
| 2004<br>[128]                | Moss          | Human<br>(10 total) | 3 – 31 months    | Pt-Ir<br>DBS          | Analysis of explanted DBS electrodes from human patients showed attachment of foreign body giant cells at each timepoint                                                                                                                |
| 2005<br>[95]                 | Biran         | Rat/5<br>(10 total) | 2 – 4 weeks      | Silicon<br>(Michigan) | The tissue response is due to the presence of the implant, not the initial injury<br>Quantified both the neuronal and glial reaction to silicon microelectrodes<br>Showed that cells attached to explanted electrodes secrete cytokines |
| 2005<br>[129]                | Spataro       | Rat/3<br>(24 total) | 1 – 6 weeks      | Silicon               | Peripheral dexamethasone injections do not affect cell attachment to the electrode                                                                                                                                                      |
| 2006<br>[130]                | He            | Rat/3<br>(9 total)  | 1 day – 4 weeks  | Silicon<br>(Michigan) | Laminin coating on electrode may decrease gliosis                                                                                                                                                                                       |
| 2007<br>[131]                | Biran         | Rat/6<br>(51 total) | 1 – 4 weeks      | Silicon<br>(Michigan) | The method of attaching an implant to the skull significantly affects the tissue response                                                                                                                                               |



**Table 1.2 cont**

| <b>Year</b><br><b>[Ref.]</b> | <b>Author</b> | <b>Species/N</b>   | <b>Timepoint</b> | <b>Electrode Type</b>                                     | <b>Contribution</b>                                                                                                         |
|------------------------------|---------------|--------------------|------------------|-----------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|
| 2007<br>[132]                | McConnell     | Rat/2<br>(6 total) | Up to 4 weeks    | Silicon<br>(Michigan)                                     | Electrodes become more integrated into target tissue over time as assessed by the extraction force necessary to remove them |
| 2007<br>[133]                | Seymour       | Rat<br>(7 total)   | 4 weeks          | Parylene-C<br>coated silicon                              | Size of implant does not correlate to the tissue response in the adjacent compartment                                       |
| 2007<br>[134]                | Zhong         | Rat/2<br>(8 total) | 1 – 4 weeks      | Silicon<br>(Michigan)                                     | Quantified the amount of chondroitin sulfate proteoglycans adjacent to the implant                                          |
| 2008<br>[135]                | Leung         | Rat/9<br>(9 total) | 1 – 12 weeks     | Silicon<br>(Michigan)                                     | Quantitative image analysis of the brain tissue response extended to a chronic timepoint                                    |
| 2009<br>[136]                | Linsmeier     | Rat/4              | 1 – 12 weeks     | GaP nanowires                                             | By 12 weeks, nanowire implants completely phagocytosed in rat brain                                                         |
| 2009<br>[137]                | McConnell     | Rat/6              | 2 – 16 weeks     | Silicon<br>(Michigan)                                     | Tissue reaction resembles neurodegenerative state                                                                           |
| 2009<br>[138]                | Ward          | Rat/3              | 2 – 31 days      | Silicon<br>(Michigan, Utah)<br>Ceramic<br>Microwire Array | Initial effort to compare available electrode designs in terms of recording ability and tissue response.                    |



**Figure 1-2.** Tissue response to recording electrodes by type. Survey of the peer reviewed literature showing studies of the tissue response to electrodes. Note that although microwires have shown superior recording longevity, and thus may be more appropriate in chronic clinical applications, the majority of the work in the field has now shifted focus to planar silicon microelectrode arrays.

functionally into an active state, home toward the site of injury, and are involved in phagocytosis and debris clearance [108]. In addition, following activation, microglia and extravasated blood-borne macrophages can release reactive oxygen species,  $\text{TNF-}\alpha$ , and NO which can damage healthy bystander cells such as neurons [139, 140]. Such proinflammatory cytokines have been detected from explanted microglia/macrophages found adhering to SiMEAs [95]. As part of the characteristic reaction of brain tissue to

implanted electrodes, activated macrophages/microglia have been reported at the electrode-tissue interface at all timepoints investigated by using immunomarkers against CD68 [119, 126, 129, 131, 134, 135]. This is in stark contrast to wound healing in the absence of a foreign body, such as a cerebral stab wound, in which resolution of the microglia/macrophage response is achieved by 2 - 4 weeks [95, 141, 142]. This difference suggests that the presence of the insoluble implant causes these cells to remain active indefinitely, in a process termed frustrated phagocytosis, similar to the presence of insoluble beta-amyloid plaques in Alzheimer's disease [143]. However, it is not known whether decreasing the surface area available for microglia/macrophage attachment, or altering binding ability by changing the outer surface chemistry, may reduce the activation of these cells, and hence the observed brain tissue response.

In the uninjured brain, astrocytes are responsible for maintaining the local cellular microenvironment, including sequestering neurotransmitters and ions necessary for normal synaptic signaling [144]. Astrocytes also control synaptic stability [145] and, in connection with endothelial cells, form the BBB. Following injury, astrocytic processes hypertrophy and often form a scar-like layer surrounding implanted materials [146]. Several groups have hypothesized that astrogliosis may underlie recording dysfunction due to the glial scar forming a high impedance barrier which surrounds implanted electrodes [123, 126, 133]. Thus, another hallmark of the brain-tissue reaction to implanted electrodes is the formation of a syncytium composed of hypertrophic astrocytes surrounding the lesion epicenter [49, 121, 123, 125, 132].

In addition to chronic microglial activation and astrocyte hypertrophy adjacent to implanted electrodes, a region of neuronal loss surrounding the implant site has been

described by several groups using multiple animal models and electrode types [93-95, 120, 133]. In these studies, neuronal loss was demonstrated by quantifying the number of neuronal nuclei or cytoskeletal components of neurons such as neurofilaments adjacent to the implant, and comparing this to control animals or uninjured areas of the cortex. Using this approach, a zone of neuronal loss extending approximately 100  $\mu\text{m}$  from the electrode interface, overlapping with the critical recording zone as has been described. In addition, oligodendrocyte loss and myelin disruption has been observed in the tissue surrounding implanted electrodes [94, 105, 118, 146], which may reduce recording ability while sparing local neuronal populations. Another point is that the greatest success in recording longevity has been associated with the use of microwires and microwire arrays; however, there are no quantitative studies detailing the tissue response to such devices. It is also of note that the foreign body response to such implanted recording electrodes overlaps with the length scale over which such devices are intended to function.

These hallmarks of the tissue reaction to implanted recording electrodes are similar to those described for other devices such as cerebrospinal shunts [147], and deep brain stimulators [148-151], and include persistent microglia/macrophage activation at the device-tissue interface, astrogliosis, and neuronal loss. The similarities in the endpoint histology suggest that the tissue response to penetrating implants in the CNS is a general feature regardless of implant type or location.

### Inflammation and Neurogenesis

Neurogenesis is the process of generating functionally integrated neurons from stem cells and progenitor cells, and occurs throughout life in both the dentate gyrus

(DG) of the hippocampus and the subventricular zone [152, 153]. Adult neurogenesis is believed to be associated with learning and memory formation [154-156], as well as recovery following injury or disease [157-159]. Common methods for assessing neurogenesis include: BrdU injections, which are added during DNA synthesis to cells in S phase of the cell cycle [160]; immunohistochemistry for Ki-67, which marks nuclei of proliferative cells [161]; and other immunohistochemical markers such as doublecortin (DcX), for a microtubule associated protein localized in the soma and axons in developing immature neurons [162]. Studies using these methods have found that adult neurogenesis decreases following exposure to glucocorticoids [163], sleep deprivation [164], stress [165], and naturally as a function of age [166, 167]. Positive regulation of neurogenesis has been observed in similar experiments following exercise [168], environmental enrichment [169], learning [170], periods of hypoxia [171, 172], as well as seizure induction [173].

Inflammation has also been shown to strongly affect neurogenesis. Using bacterial endotoxin (LPS) injections, which result in a large degree of hippocampal inflammation, the number of newborn neurons has been shown to have a strong negative correlation with activation of microglia in the same zone [174]. In addition, this decrease in neurogenesis was believed to be caused by a spike in the local concentrations of cytokines released by activated microglia, including TNF- $\alpha$  and IL-6 [175]. However, whether the inflammatory reaction caused by biomaterial implantation in the CNS is sufficient to cause changes to neurogenesis has not yet been reported.

While the hippocampal proliferative response to CNS implants has not been reported, many reports have described changes in memory and intelligence following

clinical hydrocephalic shunting and deep brain stimulator implantation. As described previously, hydrocephalic shunting is a common medical procedure used to reduce intracranial pressure due to the accumulation of abnormal amounts of cerebrospinal fluid in patients with hydrocephalus and spina bifida, and results in similar inflammation and a chronic foreign body response as implanted electrodes. Multiple reports in children and adults have described reduced performance IQ and memory impairment following hydrocephalic shunting [176-178], which may be related to the inflammatory effects of biomaterial implantation on neurogenesis. The implantation of stimulating electrodes has been used to treat the symptoms associated with Parkinson's disease [20], depression [21], and is being investigated for use in treating obsessive-compulsive disorder [22] and epilepsy [23]. Animal models of stimulating electrodes have shown that cognitive impairments may be due to the presence of the electrode or the presence of nonpenetrating head plugs without an electrode implant [179], suggesting that the inflammation associated with the presence of any implant may impair neurogenesis. While the use of DBS may relieve symptoms, evidence in the literature suggests that, like hydrocephalic shunting, DBS also causes cognitive impairments in patients following implantation [180-183], suggesting that decreases in memory and intelligence may be a general feature of penetrating brain implants, regardless of implant type or location. In addition, HIV-associated dementia, which has a strong inflammatory component, and has become the leading cause of dementia in individuals aged 20 to 59 years, indicative of another link between inflammation and cognition [184, 185].

## CHAPTER 2

### TISSUE RESPONSE TO CHRONICALLY IMPLANTED MICROWIRES IN RAT CORTEX

#### Introduction

Cortically implanted microelectrode arrays are used for assessing neuronal activity. One such example is the microwire array, which has been widely used as a basic science tool, and has the potential to contribute to clinical device development [28, 29, 83]. However, following implantation, chronic single unit recording and indeed, recording performance in general, is inconsistent from animal to animal with this class of devices [28-30, 49-52, 92]. Available evidence suggests that the reaction the brain mounts against implanted microelectrode contributes to recording instability, however, the factors that influence the brain tissue response to implanted electrodes remain unclear.

Evidence gathered over the last 50 years indicates that the brain tissue response to penetrating electrodes consists of several major features that are observed regardless of electrode design, species studied, or implantation method, and is similar in many respects to the foreign body response in other soft tissues [50, 105]. In particular, by 4 weeks following implantation, the reaction has a stereotypical organization consisting of hypertrophic astrocytes, fibroblasts, and meningeal cells [93-95, 105, 106, 119, 121, 123,

125, 126, 129, 133, 134, 186] that surrounds an activated macrophage/ microglial/ foreign body giant cell core found immediately adjacent to the implant [95, 106, 123]. Within this region of reactive gliosis, numerous investigators have described a decrease in the neuronal population [93-95, 105, 106, 119, 120, 133, 134, 186]. Quantitative studies have shown that the response is limited to the immediate vicinity of the electrode extending a few hundred microns from the interface [95, 131]. While many of these features are observed at longer timepoints [94, 95, 105, 119-121, 126, 129, 134, 186], quantitative studies that examine the spatial distribution of relevant biomarkers as a function of indwelling time are lacking.

Toward this end, we employed a quantitative immunohistochemical approach to describe the spatial distribution of established cell type specific markers associated with the foreign body response at 2, 4, and 12 weeks following the implantation of a microwire electrode in rat cortex. A single microwire electrode was chosen as a point of comparison with other such studies that have examined the brain tissue response to a single penetrating planar electrode array [95, 123, 126, 131, 134, 135]. Specifically, the study was designed to address several questions including: a) Does inflammation persist around an implanted microwire electrode?; b) Does the pattern of reactive astrogliosis change as a function of the indwelling period?; and, c) Does the distribution of viable neurons at the device tissue interface change over the indwelling period? That is, is there evidence of progressive neuronal degeneration?



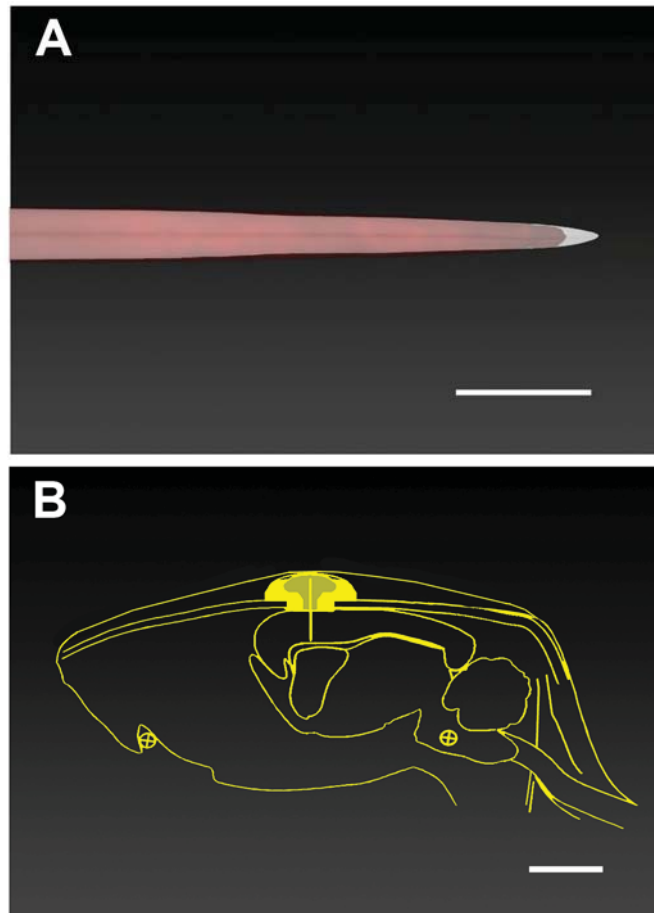
## Materials and Methods

### *Microelectrodes*

Single shank, stainless steel microwires were provided by FHC (Bowdoinham, ME). Microwires had a 75  $\mu\text{m}$  diameter shank with a sharp conical tip, approximately 1  $\mu\text{m}$  in diameter, and were 5 mm in total length. The microwires were coated by the manufacturer with Epoxylite insulation, except for the last 25  $\mu\text{m}$  of the tip which constituted the recording site, as is shown in Figure 2-1 A. All microwire electrodes were cleaned by immersion in 70% ethanol and rinsed several times in sterile DI water, followed by sterilization with ethylene oxide (EtO). Following EtO treatment, microwires were allowed to outgas for at least 48 hours prior to implantation.

### *Animal surgery*

All procedures involving animals were conducted in accordance with the University of Utah Institutional Animal Care and Use Committee (IACUC). Adult male Sprague Dawley rats (200-225 g) were anesthetized via an intraperitoneal injection of ketamine (65 mg/kg), xylazine (7.5 mg/kg), and acepromazine (0.5 mg/kg). After reaching a full level of anesthesia as assessed by tail/toe pinch, the animals' heads were shaved, and the scalp was disinfected with 70% isopropanol and betadyne. Animals were then transferred to a stereotaxic frame, and a midline incision extending the length of the skull was made. The skin was retracted and the fascia was removed with a scalpel blade and then dried with cotton tipped applicators. A 3 mm diameter burr hole was cut through the skull by a pneumatically-driven trephine under PBS irrigation, with the center of the hole positioned +0.2 mm forward of bregma, and 3 mm lateral to bregma.



**Figure 2-1.** Electrode and implantation schematic. A) Bright field image of a representative stainless steel microwire electrode used in the study insulated with Epoxylite 600M, along the shank, with the last 25 $\mu$ m of the tip uninsulated. Average width = 75  $\mu$ m; surface area (2 mm implant) = 0.46 mm<sup>2</sup>. Scale bar 100  $\mu$ m. B) CAD drawing of a mid sagittal section of rat head showing location of implant and anchoring polyurethane grommet. All microwires were lowered stereotactically to a controlled depth of 2 mm below the surface of the cortex, and attached to a soft polyurethane grommet using a UV-curable adhesive as shown. Scalebar = 5 mm.

Using a stereomicroscope, the exposed dura was then opened with a 21 G needle. The microwire was implanted as shown schematically in Figure 2-1 B. A custom fabricated polyurethane grommet was placed in the center of the burr hole. The microwire was then lowered with a manual fine tooth stereotactic drive through the open lumen of the grommet to a depth of 2 mm from the surface of the cortex. The microwire was then fixed into place through the inner lumen of the grommet using a UV curable medical grade adhesive (MD 1181-M, Dymax, Torrington, CT). After the adhesive cured, the leads extending above the polyurethane port were cut using fine surgical scissors, the scalp incision was closed with 5/0 silk sutures and the animals were allowed to recover.

#### *Euthanasia and tissue preparation*

At 2, 4 and 12 weeks postimplantation (n=9 animals, each timepoint), animals were terminally anesthetized via an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (30 mg/kg), and perfused transcardially with PBS followed by 4% paraformaldehyde, at a volumetric flow rate of 50 ml/min. The microwire-grommet system was carefully retrieved with fine surgical scissors and microdissection forceps, followed by removal of the brain. Brains and microwire electrodes were postfixed separately at 4°C with 4% paraformaldehyde for 24 hours. Following postfixation, brains were serially sectioned in the horizontal plane (n=6, each timepoint) at 40 µm thickness on a vibratome, to a depth of 2.9 mm (72 sections per animal), or serially sectioned in the coronal plane (n=3, each timepoint) through the electrode-tissue interface to a distance of 1 mm in each direction (48 sections per animal).

### *Immunohistochemistry*

Three out of every four serial sections were batch-treated with antibodies against GFAP (2.4 µg/ml, DAKO, Carpinteria CA), for an intermediate filament specific to astrocytes, and the three consecutive sections were alternately immunoreacted with antibodies against CD68 (ED-1, 0.5 µg/ml, AbD Serotec, Raleigh, NC) for activated microglia/ macrophages, NF-160 (1.0 µg/ml, Sigma-Aldrich, St. Louis, MO) for neuronal processes, and NeuN (2.0 µg/ml, Millipore, Billerica, MA) for neuronal nuclei. Each fourth section was left for additional markers, including MAP-2 (2.0 µg/ml, Millipore, Billerica, MA) for dendrites, Myelin Basic Protein (MBP, 2.5 µg/ml, Abcam, Cambridge, MA) for myelin and oligodendrocytes, and anti-rat IgG (2.0 µg/ml, Southern Biotech, Birmingham, AL) to assess blood-brain-barrier (BBB) integrity. Antibodies were diluted in a blocking solution consisting of 4% (v/v) goat serum (Invitrogen, Carlsbad CA), 0.5% (v/v) Triton-X 100, and 0.1% (w/v) sodium azide. Tissue sections were batch treated for 1 hour in a blocking solution at room temperature, followed by incubation with primary antibodies overnight at 4°C. After three rinses in PBS at room temperature to remove excess antibody (1 hour/each rinse step), appropriate fluorescently-labeled secondary antibodies were applied in block for 1 hour at room temperature, followed by three washes in PBS (1 hour/each rinse step). All sections were also counterstained with 10 µM DAPI for cell nuclei. Tissue sections were mounted on microscope slides, covered with Fluormount-G (Southern Biotech), and coverslipped. Explanted electrodes were immunostained for GFAP and CD68 in a similar manner as tissue. Immunohistochemistry also included nonimplanted control animals (n=5), and primary controls in which the primary antibodies were omitted.

### *Quantitative intensity analysis*

Fluorescent images of tissue sections from Layers V and VI of the cortex, near the tip and recording site of the microwires, were captured with a Coolsnap digital camera and a Nikon Eclipse E600 microscope, using identical exposure times and conditions which were optimized for each immunomarker. In addition, some sections were imaged at higher magnifications using a Zeiss confocal microscope. Lightfield corrections and background subtraction using primary controls were performed prior to line profile analysis. The fluorescent intensity was quantified, averaged, and compared using a custom LabView-based image analysis program (National Instruments) that takes a circular array of line profiles originating from the implant site and extracts the fluorescent intensity as a function of distance from the implant site.

For each section, line profiles were extended through the section originating at the electrode-tissue interface, at a spacing of  $\sim 10^\circ$  between each line profile (approximately 35-40 profiles per image). At each point along the lines, an anti-alias pixel extraction algorithm was used to derive the pixel intensity value [127]. A mean intensity profile was then calculated by averaging all of the line profiles per section. The average intensity for each immunomarker was then combined with all the sections for each timepoint, to obtain an average intensity profile for the cohort. In order to quantify the changes in neuronal nuclei, images treated with antibodies for NeuN were overlaid with a 50  $\mu\text{m}$  circular grid, originating at the electrode-tissue interface, and the number of NeuN<sup>+</sup> and DAPI<sup>+</sup> nuclei in each grid were counted along a 50  $\mu\text{m}$  wide section of the image in the medial-lateral and posterior-anterior directions. The number of neuronal

nuclei counted was then divided by 4 to determine the average number of nuclei per 50 x 50  $\mu\text{m}$  bin.

In addition to line profile analysis, to check for reaction symmetry, surface plots of each image were created with Image Pro Plus. Each antibody was analyzed separately, and an average plot was created for each.

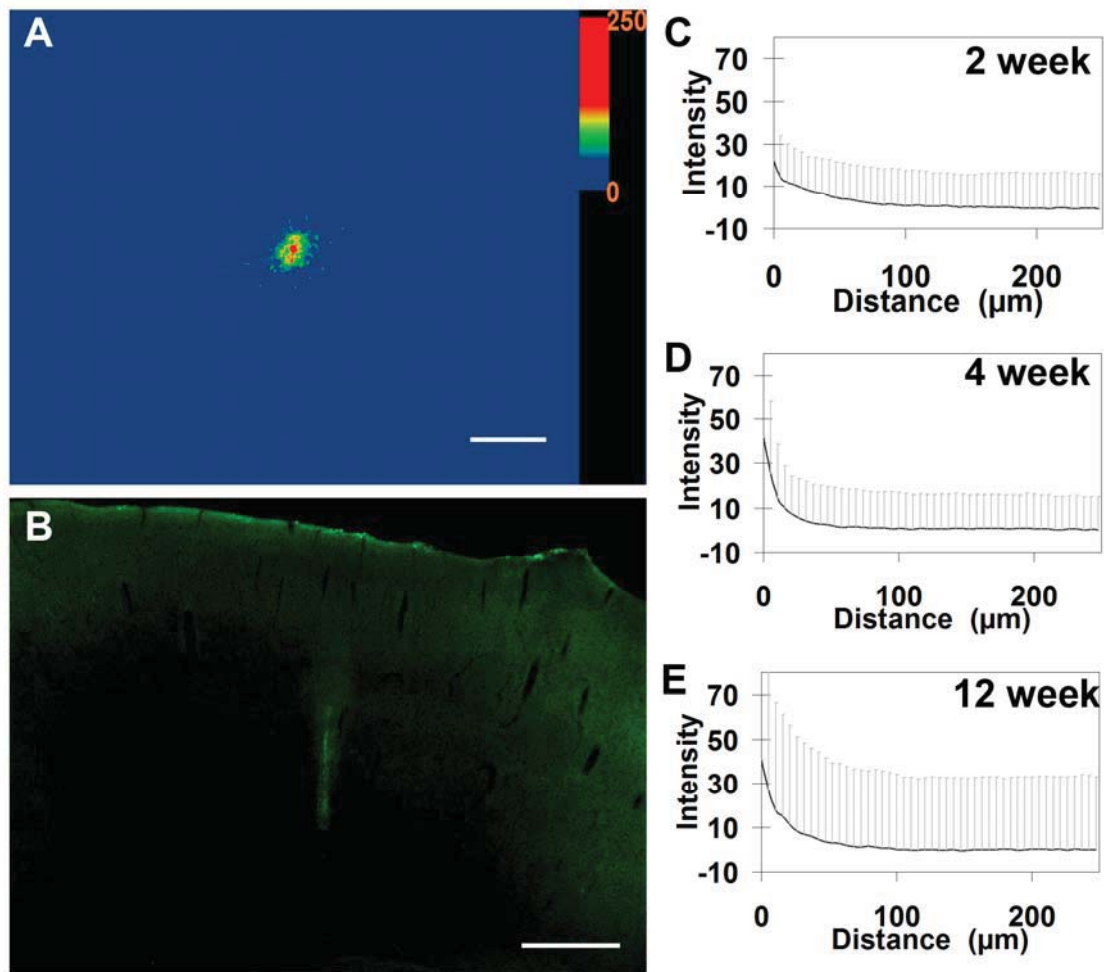
### *Statistical analysis*

The area under the curve of each average intensity profile for each immunomarker at 2, 4 and 12 weeks in the first 100  $\mu\text{m}$  adjacent to the electrode, corresponding to the critical recording zone, was compared across timepoints using ANOVA and a Tukey posthoc test. Areas under the curve were analyzed using an SPSS software package, with significance considered at  $p < 0.05$ .

## Results

### *Quantitative glial and neuronal response*

No attached cells were observed on explanted microwires at any timepoint studied (data not shown). The activated microglia/macrophage response to implanted stainless steel microwires consisted of punctate CD68<sup>+</sup> immunoreactivity localized adjacent to and within the electrode track. The CD68 immunoreactive zone was circular in shape and reflected the geometry of the implanted electrode, and was symmetric along the entire electrode shaft (Figure 2-2 A). There was also a large number of DAPI<sup>+</sup> cells within the electrode track in each animal investigated. No evidence of microglia/macrophage activation was observed in other more distant brain areas (Figure 2-2 B). The average CD68 intensity as a function of distance over the first 250  $\mu\text{m}$  from the electrode tissue

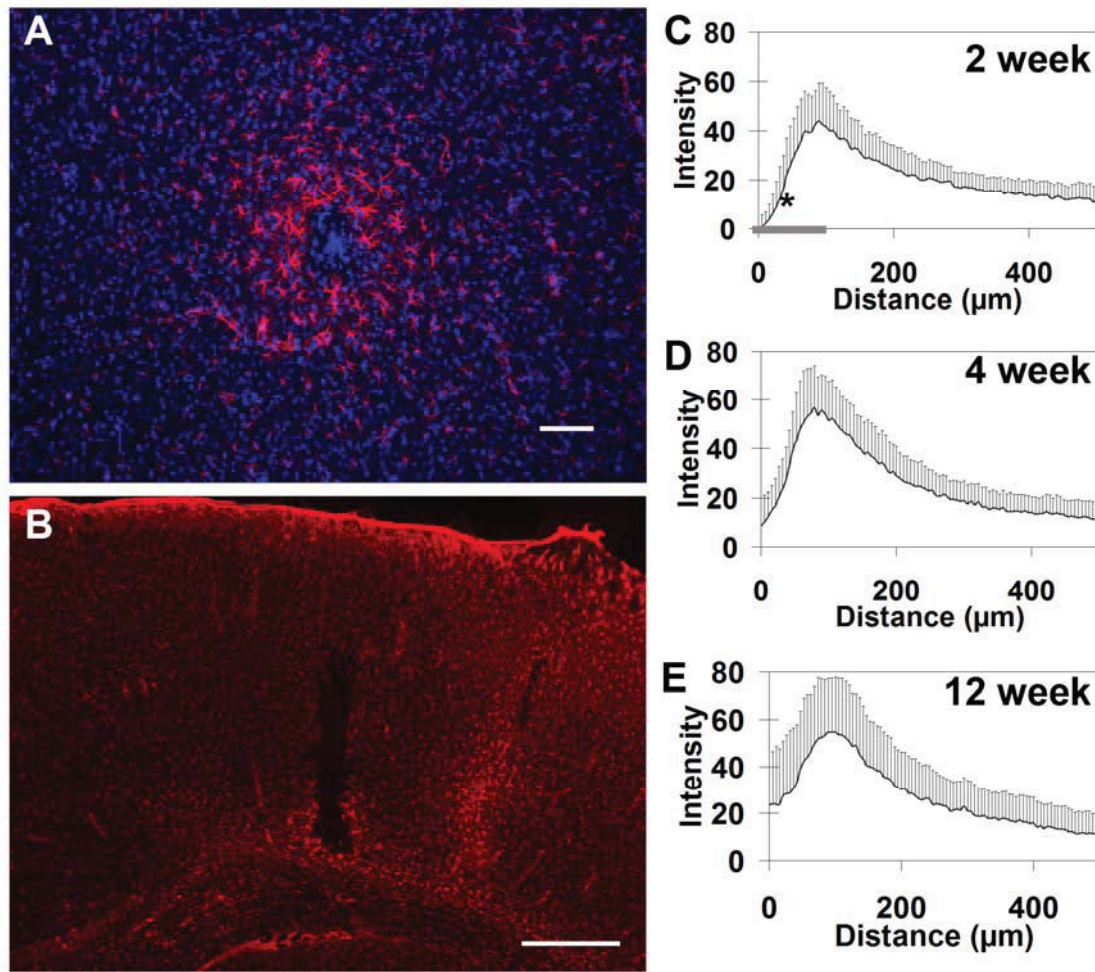


**Figure 2-2.** CD68 immunoreactivity. A) Average 12 week surface plot for CD68 showing symmetric distribution of immunoreactivity directly adjacent to the implanted microwire which was circular in shape and mirrored the geometry of the implanted electrode. B) Representative coronal section at 12 weeks treated with anti-CD68 showing that the increase of this biomarker is only localized to the electrode track. Average ED1 as a function of distance from the implant interface  $\pm$  standard deviation C) 2 weeks following implantation D) after 4 weeks, and E) after 12 weeks. In B – D, no differences were found in the area under the curve of the recording zone (100  $\mu$ m) between timepoints. Scalebar in A = 100  $\mu$ m, B = 500  $\mu$ m.

interface at 2, 4, and 12 weeks is shown in Figures 2-2 C – E, respectively. At 2 weeks, the CD68 immunoreactivity was spread further from the interface than at later timepoints. By 4 and 12 weeks, the CD68 immunoreactivity was confined in the zone directly adjacent to the electrode-tissue interface. Quantitative analysis of the area under the curve showed no statistical differences in the first 100  $\mu\text{m}$  across timepoints. In addition, the variability associated with the CD68 mean fluorescent signal (Figure 2 C-E) increased at the later timepoints.

A diffuse, hypertrophic GFAP<sup>+</sup> immunoreactive zone surrounded the CD68<sup>+</sup> immunoreactivity at each timepoint (Figure 2-3 A). We observed a zone of cells that were not immunoreactive for GFAP or CD68 between the CD68<sup>+</sup> layer and the GFAP<sup>+</sup> layer at 2 and 4 weeks. This zone was filled with nuclei as indicated by a positive DAPI reaction (Figure 2-3 A). In addition, similar to CD68, the GFAP<sup>+</sup> immunoreactive zone was circular and reflected the surface geometry of the implanted electrode, with a circular cross-sectional geometry in horizontal sections and a cylindrical cross-sectional geometry in coronal sections (Figure 2-3 B). Quantitative analysis of GFAP<sup>+</sup> immunoreactivity at 2, 4, and 12 weeks is shown in Figures 2-3 B – D. At 2 weeks, immunoreactivity directly adjacent to the electrode-tissue interface was decreased compared to nonimplanted control animals. Immunoreactivity increased at 4 and 12 weeks adjacent to the electrode. Area under the curve analysis in a 100 micron zone surrounding the electrode revealed a statistically significant decrease in GFAP immunoreactivity at 2 weeks as compared to later timepoints. Similar to CD68, the variability associated with the GFAP immunoreactivity increased across the cohort as the indwelling period increased.





**Figure 2-3.** GFAP immunoreactivity. A) Representative 12 week horizontal section illustrating the hypertrophic astrocyte response with GFAP immunoreactivity. DAPI (blue) indicates the presence of additional cells directly adjacent to the electrode. Note the lack of interdigitating GFAP<sup>+</sup> processes surrounding the implantation tract. B) Representative coronal section at 12 weeks showing GFAP immunoreactivity. Average GFAP immunoreactivity as a function of distance from the implant interface  $\pm$  standard deviation C) 2 weeks following implantation D) after 4 weeks, and E) after 12 weeks. The area under the curve of the recording zone was statistically different at 2 weeks from the later timepoints ( $p \leq 0,05$ ). Scalebar in A = 100  $\mu$ m, B = 500  $\mu$ m.

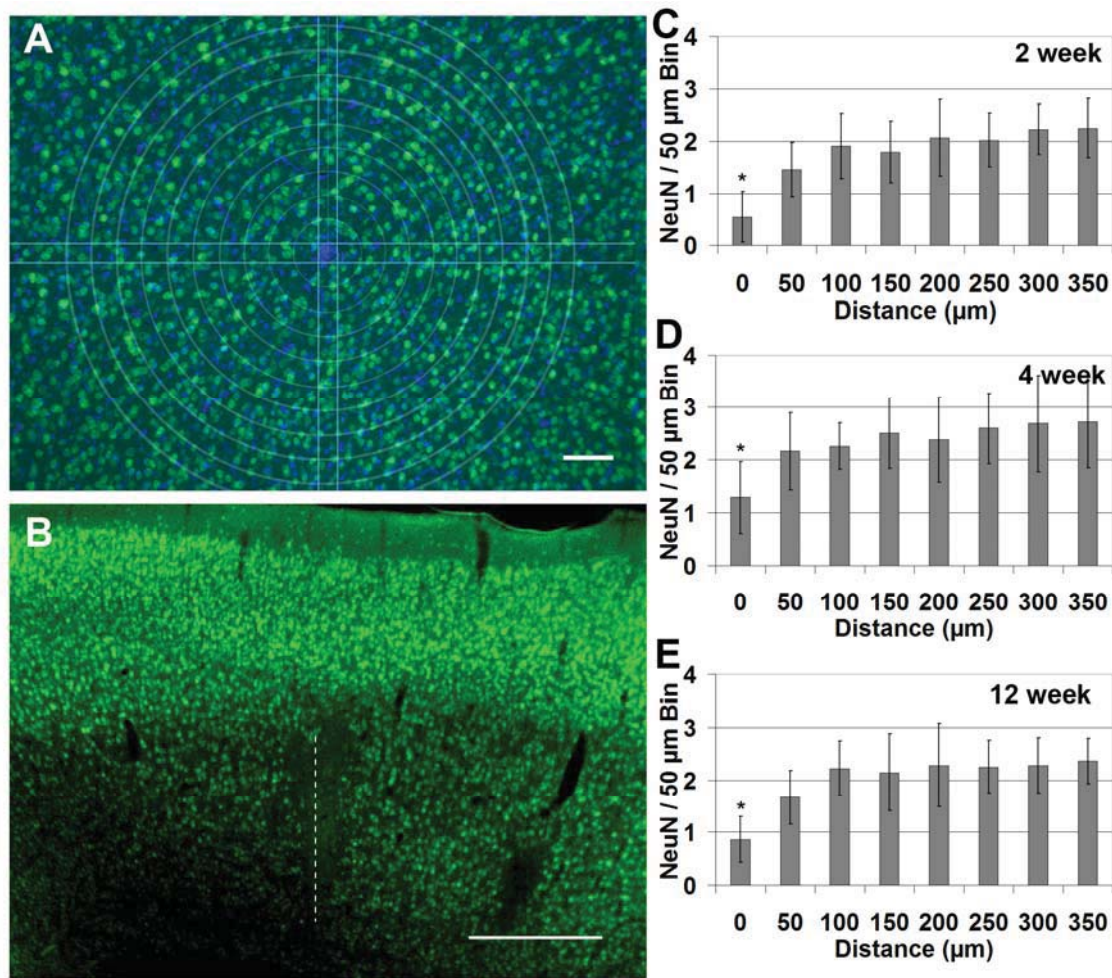
A reduction in neuronal nuclei was observed within a zone approximately 50  $\mu\text{m}$  from the electrode tissue interface as assessed with NeuN. Figure 2-4 A shows the typical NeuN phenotype at 4 weeks overlaid with the grid used for counting. The results of the quantitative analysis at 2, 4, and 12 weeks are shown in Figures 2-4 C – E. Compared to uninjured tissue, there was a statistically significant reduction in NeuN immunoreactivity within a 50  $\mu\text{m}$  zone from the electrode-tissue interface at each time point compared to uninjured tissue. There was no reduction of neuronal nuclei at greater distances from the electrode-tissue interface at any time points or in other areas of the brain (Figure 2-4 B).

To assess the state of the blood brain barrier, sections were randomly selected for reaction with antisera against rat IgG. We observed immunoreactivity for rat IgG adjacent to and along the implantation tract in animals at each timepoint, which was variable from animal to animal and variable along the length of the electrode tract (Figure 2-5 A and B).

Additional sections were immunoreacted with MBP, a protein found both in myelinated axons and oligodendrocytes. A reduction in MBP immunoreactivity was observed (Figure 2-5 C and D) that extended the length of the electrode whose distribution appeared to mirror the surface geometry of the implanted microwire.

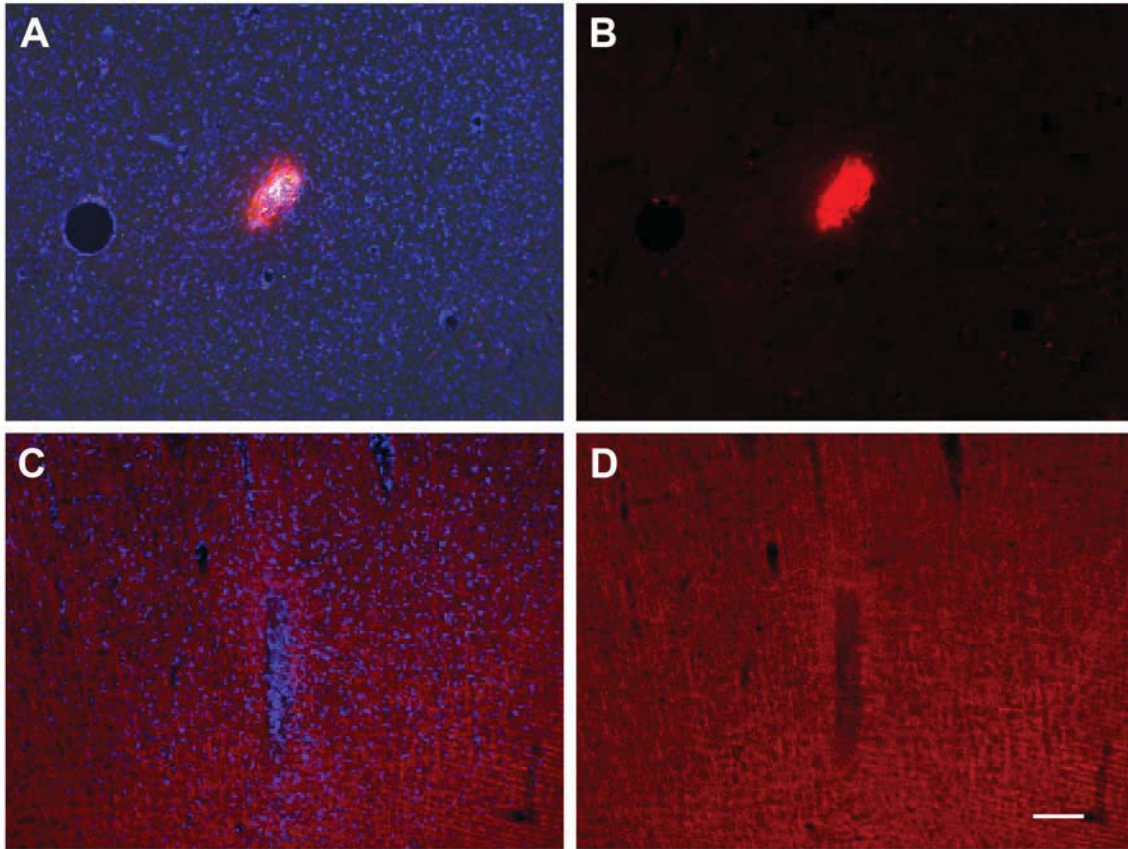
### *Neuronal processes*

As has been shown in previous studies, a region of decreased neuronal filament immunoreactivity was observed surrounding the implantation tract. In order to determine whether the observed decrease was due to a loss in axons or dendrites, we used MAP-2 antisera (Figure 2-6), a biomarker for dendrites, to assess spatial distribution of dendrites

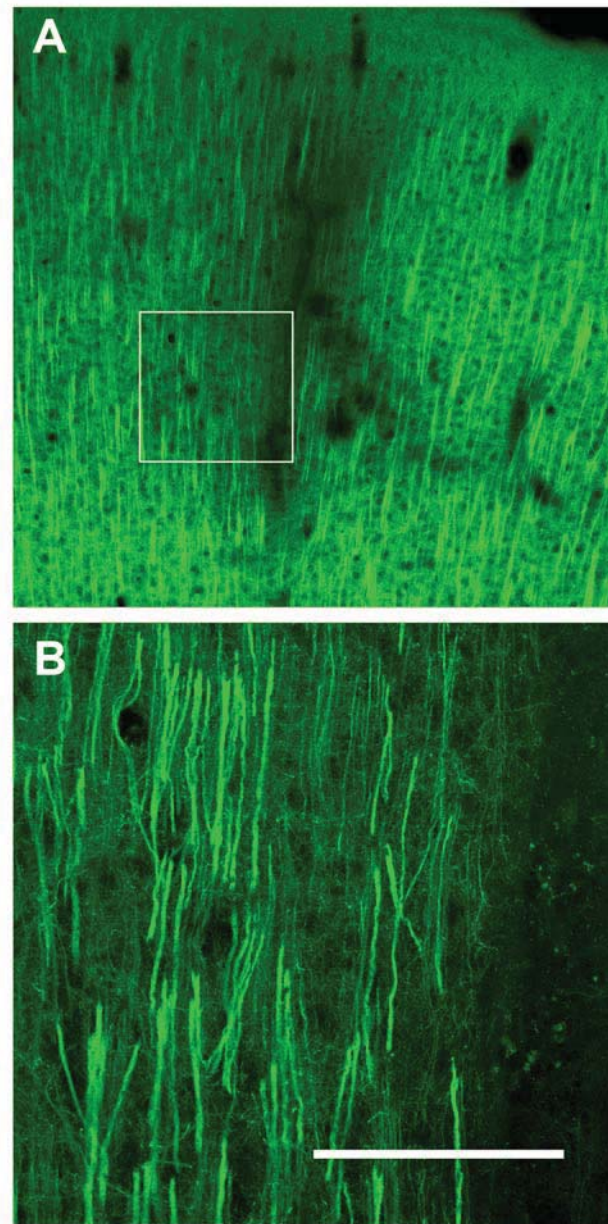


**Figure 2-4.** NeuN immunoreactivity. A) Representative 12 week NeuN image, overlaid with the grid for quantifying the number of neuronal nuclei, illustrating the typical minor reduction in nuclei. B) Representative coronal section adjacent to the electrode tract 12 weeks after implantation showing a reduction of NeuN immunoreactivity. Average number of NeuN + nuclei as a function of distance from the implant interface  $\pm$  standard deviation C) 2, D) 4, and E) 12 weeks after implantation. There is a significant decrease in the number of NeuN+ nuclei in the first 50  $\mu$ m next to the microwire, as compared to uninjured tissue. In each graph, the average number of NeuN<sup>+</sup> nuclei in nonimplanted controls is shown in light gray. (\* $p \leq 0.05$ ). Scalebar in A = 100  $\mu$ m, B = 500  $\mu$ m.





**Figure 2-5.** IgG and Myelin Basic Protein immunoreactivity. A) Representative 12 week horizontal section showing immunoreactivity for CD68 (green), and DAPI (blue) showing distribution of cell nuclei. B) IgG immunoreactivity was observed principally surrounding the electrode tract, indicating leakiness of the BBB. Same section as A showing only IgG. C) Representative 12 week coronal section illustrating a reduction of myelin basic protein immunoreactivity in a section immediately adjacent to the electrode (red) within the lower cortex. Addition of the DAPI (blue) channel shows this MBP nonimmunoreactive zone to be filled with cells. D) MBP immunoreactivity from C shown alone. Scalebar = 100  $\mu$ m.



**Figure 2-6.** MAP- 2 immunoreactivity. A) Representative coronal confocal image in a section adjacent to the implanted microwire 12 weeks after implantation showing a decrease in cortical dendrite immunoreactivity that had the shape of an inverted pyramid, with more of a reduction in MAP-2 signal observed in the upper cortex than at deeper portions of the cortex. B) Detail from A showing the reduction of MAP-2<sup>+</sup> processes on the far right of the panel, Scalebar 100  $\mu$ m.

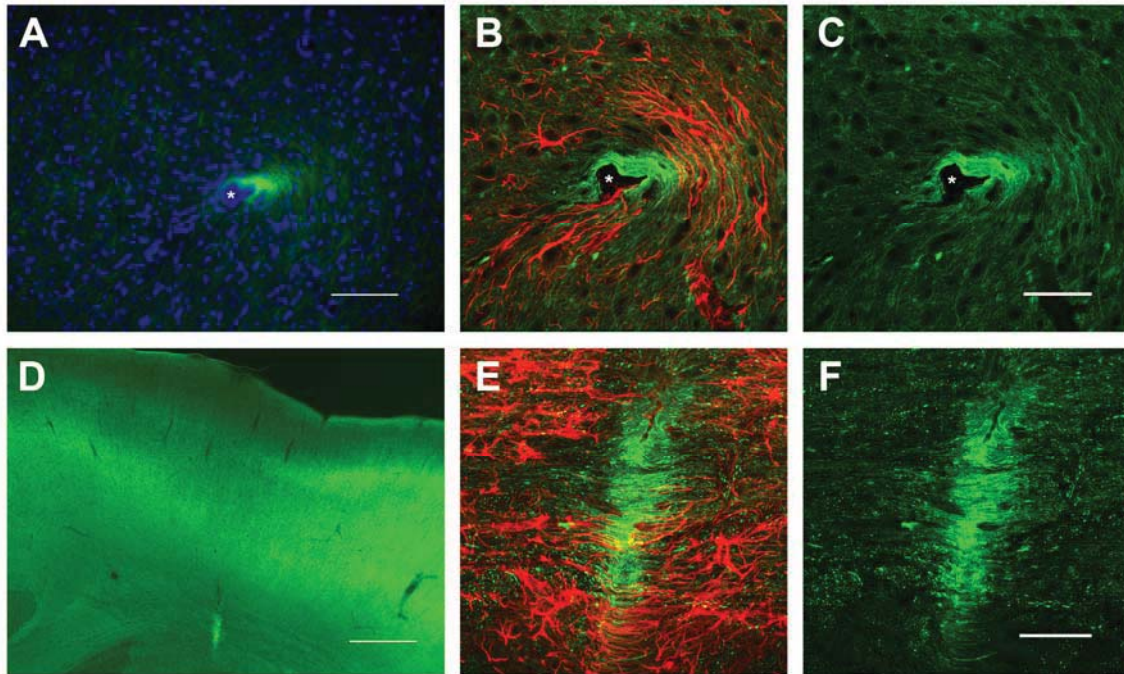
near the implantation tract. We consistently observed a reduction in MAP-2 immunoreactivity that extended approximately 100  $\mu\text{m}$  from the implantation tract, whose distribution also mirrored the surface geometry of the implanted electrode, which did not appear to change as a function of the indwelling period (Figures 2-6 A and B).

In many of the animals, we observed an increase in immunoreactivity for NF-160 directly adjacent to the implantation tract, which was asymmetrically distributed in horizontal section (Figures 2-7 A and D). This pattern of increased NF immunoreactivity was observed only in sections deep within the cortex, approximately 1.5 to 2 mm from the top of the cortex. The increase in NF-160 immunoreactivity was observed more frequently at later timepoints including: 2 weeks (3 out of 9 animals); 4 weeks, (8 out of 9 animals); and, 12 weeks, (all 9 animals). In order to more fully understand the specificity of the immunoreactive signal, we imaged selected sections using confocal microscopy (Figures 2-7 B and C). The pattern of NF-160 immunoreactivity appeared to be fibrous in structure, resembling axons but brighter and hypertrophic compared to the pattern observed in uninjured animals and was always adjacent to GFAP+ filaments. Figure 2-7 B shows the neuronal filaments and GFAP surrounding the implant site, and Figure 2-7 C shows the NF-160 immunoreactivity alone, depicting the upregulation directly adjacent to the microwire. Figures 2-7 D, E, and F show this phenotype in coronal sections.

### Discussion

The results of our study show that inflammation persists around a single microwire electrode over a three month indwelling period in rat cortex. At no point in time or in any of the 27 implanted animals was the implantation tract free of ED1 immunoreactivity. We consistently observed the pan-macrophage marker, CD68, at 2, 4





**Figure 2-7.** Regeneration. NF-160 immunoreactivity was enhanced in the deep cortex near the electrode tip. A) Representative 12 week horizontal section showing NF-160 immunoreactivity (green) and DAPI+ cell nuclei (blue) near the implantation tract (center of image). B) Same image as A, obtained using a confocal microscope to show NF-160<sup>+</sup> (green) and GFAP<sup>+</sup> (red) immunoreactivity processes surrounding the implantation tract (center of image). C) NF-160<sup>+</sup> immunoreactivity as shown in B alone. D) Representative 12 week coronal section showing NF-160<sup>+</sup> at the deepest level of the cortex. E) Confocal image of the region of enhanced NF immunoreactivity in D showing the relationship of NF-160 (green) immunoreactivity and GFAP (red) immunoreactivity directly below the electrode tip. F) NF-160 immunoreactivity of E alone. In A– C, electrode implantation site is shown with an asterisk. All scalebars = 100  $\mu$ m.

and 12 weeks following implantation along the electrode tract and near the recording tip that was distributed more or less uniformly along the implant surface. We observed no significant increases in the distribution of CD68 along the dorsoventral axis of the microwire, suggesting that inflammation was not exacerbated near the tip or along the insulated portions of the electrode. CD68 immunoreactivity observed at 2 weeks was spread over a larger brain region surrounding the implant site compared to 4 and 12 weeks, suggesting more regional macrophage trafficking near the implant shortly after implantation that then became more tightly associated with the implant interface. The CD68<sup>+</sup> immunoreactivity observed correlates to earlier studies that used histochemical methods and observed large numbers of microglia next to chronically implanted stainless steel electrodes placed in cat brain [94]. Several human postmortem studies investigating the tissue reaction to implanted DBS electrodes over indwelling periods from 2 months to several years also have reported activation of microglia/macrophages in the tissue surrounding implanted DBS electrodes, as well as, attached to retrieved DBS electrodes [122, 124, 128, 148-151, 187, 188]. Together the results suggest that activated microglia/macrophages remain adjacent to implanted microwires, as well as other electrode system, for the life of the implant [189].

In this study, we also provide evidence that the spatial distribution of reactive astrogliosis surrounding a microwire electrode as determined by GFAP<sup>+</sup> immunoreactivity does not increase as a function of time over a 3 month indwelling period in rat cortex. It therefore appears unlikely that reactive astrocytes progressively push neurons away from the recording zone as has been suggested as a failure mechanism for single unit recording [52]. Extensive astrocyte encapsulation as determined by an



interdigitation of GFAP<sup>+</sup> immunoreactivity also was not observed in our study; rather, a zone of decreased GFAP<sup>+</sup> immunoreactivity was observed surrounding the implant during the first 4 weeks.

The spatial distribution of GFAP<sup>+</sup> immunoreactivity did, however, change over time. The peak of relative intensity got closer to the implant surface as a function of time, perhaps indicating contraction of the gliotic scar. Others have reported similar findings using insulated stainless steel wire electrodes in cat cortex which appeared to contract between 2 and 4 weeks and remained constant thereafter [105]. In the uninjured brain, astrocytes are responsible for maintaining the local cellular microenvironment within the brain [144], as well as controlling synaptic stability [145]. It is unclear what the biological significance of the GFAP- region is that we observed immediately next to the electrode, especially when one considers that GFAP is believed to only be present in approximately 15% of astrocytic processes [190]. It is possible the tissue surrounding the implant may still be in contact with astrocytic processes and be neurophysiologically intact.

In this study, we also show that the spatial distribution of neuronal cell bodies as measured by NeuN<sup>+</sup> immunoreactivity does not change as a function of the indwelling period, but remains stable from 2 to 12 weeks following microelectrode implantation. A reduction in NeuN<sup>+</sup> immunoreactivity was restricted to an arc of 50  $\mu\text{m}$  surrounding the electrode, indicating that any neuronal loss was a localized interfacial phenomenon. Though we found no evidence of progressive neuronal loss in our model, we acknowledge that continued loss and replacement may occur adjacent to more complex implanted high density arrays which produce multiple penetrating injuries and have

extensive surface area at the base of the array which contacts brain tissue or in cases where leachable agents may be involved in electrode packaging [191, 192]. A reduction of immunoreactivity of neurofilaments surrounding the electrode has been described adjacent to planar silicon microelectrode arrays [95], but in this study we observed only a reduction in the dendritic marker MAP-2, while NF-160 was enhanced in the deep cortex in most animals. .

In a search for other components of the tissue reaction which may underlie chronic single unit recording inconsistency, we investigated biomarkers associated with oligodendrocyte health and blood brain barrier function (BBB). Myelin loss is a hallmark of such diseases as multiple sclerosis and progressive multifocal leukoencephalopathy that have a neuroinflammatory component, and has been observed in response to electrode implantation [105]. A reduction in MBP<sup>+</sup> immunoreactivity was observed in the tissue surrounding the implanted electrode, which is consistent with loss of myelin. This may be related to the reduction in the neuronal cell bodies near the electrode tract or may be progressive. Unfortunately we did not have enough sections to perform a quantitative analysis and the state of myelin as a function of time remains to be elucidated. We examined the distribution of IgG as an indicator of BBB integrity as IgG is normally excluded from the extracellular space of normal brain tissue [193, 194], but is found in brain tissue following injury and inflammation [195]. Our results indicate a leakiness of the BBB in the area surrounding the electrode, which is likely associated with macrophage trafficking to and from local vasculature in response to local cytokines like MCP, which has been shown to be secreted by macrophages and activated microglia associated with implanted microelectrode arrays [95]. Changes in BBB permeability

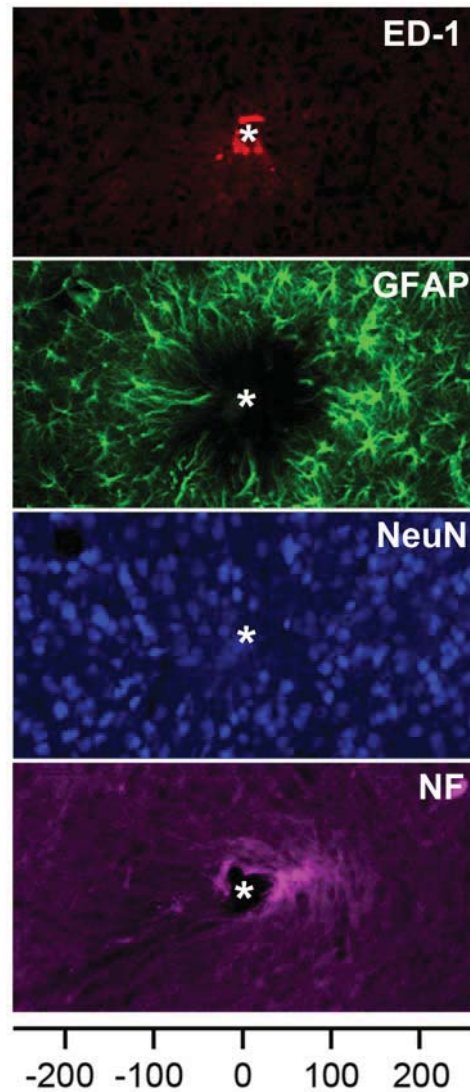
have the potential of changing the local extracellular ionic milieu, which may contribute to chronic recording inconsistency by altering ionic homeostasis in the local microenvironment. Due to the large number of sections used for quantitative analysis, we were unable to quantify the spatial changes for IgG as a function of indwelling period but our assessment suggested that it was a common observation at the point of contact of the electrode and brain tissue and was increased in places where small blood vessels were close to the implant surface.

As compared to our previous report of the brain tissue response to implanted silicon microelectrode arrays [95], we observed an overall reduced tissue reaction to the microwire electrode. Our results show a reduction in the spatial distribution of CD68 immunoreactivity at the electrode-tissue interface, a reduced GFAP response, less of a change in neuronal cell bodies, and an increase in neurofilaments near the recording tip (Figure 2-8). If one assumes that the amount of surface presented is proportional to the number of inflammatory cells that can interact with such a surface, this is an unexpected finding since the surface area of the microwire electrode is approximately double that of the planar silicon microelectrode used in our previous studies (0.46 mm<sup>2</sup> vs. 0.25 mm<sup>2</sup>) [95, 131, 135]. Clearly other factors are involved.

Some of the differences may be due to surface curvature or to the geometry of the microwire or its surface coatings. The *in vitro* reaction adjacent to cylindrical biomaterials recently has been described [196, 197], in which it was shown that brain-derived neurons exhibit neurite outgrowth that is influenced by substrate curvature, showing increased axonal outgrowth along the long axis of a cylindrical geometry. This directionality bias was shown to increase as cylindrical diameter decreased. In the

current study, as the diameter of the microwire decreased toward the tip, the relative intensity of NF-160<sup>+</sup> fibers increased adjacent to the electrode. In addition, Sorenson et al. [198] showed that neurite orientation was aligned by an underlying astrocyte layer in contact with a grooved substrate. Similarly, the astrocyte processes we observed were in close contact with the microwire, and may have provided directional cues which caused adjacent cells to orient in a circular pattern [199].

A surprising observation was the increases in NF-160<sup>+</sup> immunoreactivity observed only near the distal tip of the implanted electrode, which increased with frequency at later timepoints. Evidence of regenerating axons adjacent to chronically implanted microelectrodes has been reported adjacent to stainless steel electrodes in the past [94, 105]. The increase in neurofilament density was in contrast to our observations near implanted silicon microelectrode arrays [95], which may be explained by the shorter end point of that study or perhaps alterations in the balance of macrophage secreted factors. Clearly, a more detailed investigation is required to elucidate the importance of our observations. A summary of the tissue response to microwires at 12 weeks is shown in Figure 2-8.



**Figure 2-8.** Summary of the tissue response at 12 weeks. These images illustrate the average spatial distribution of foreign body response to a single microwire at the recording tip, which consists of a core of ED1 immunoreactivity mostly 1 to 2 cell layers wide surrounded by diffuse but hypertrophic GFAP<sup>+</sup> processes. In the zone of reactive gliosis neuronal nuclei were reduced and immunoreactivity for NF-160 at the microwire-tissue interface. The position of the microwire is illustrated by the asterisk. Images were pseudocolored for illustration.

## CHAPTER 3

### A COMPARISON OF THE BRAIN TISSUE RESPONSE TO IMPLANTED PARYLENE-C COATED AND UNCOATED PLANAR SILICON MICROELECTRODE ARRAY

#### Introduction

Implanted recording electrodes have aided our understanding of the functional organization and neurophysiological workings of the mammalian nervous system, and appear, based on evidence to date, to become increasingly used in a variety of healthcare applications that will improve the quality of life of those affected with CNS related disabilities [27, 33]. What has been shown is that neural signals can be recorded from different brain regions by a number of different technologies in a variety of species for periods of time extending from months to well over a year that can be interpreted and used to control a computer or robot or prosthetic device [29, 74, 81, 82]. With the feasibility firmly established for various brain computer and brain machine interfaces, the focus has shifted to understanding how to maintain consistent, long-term operation of the implanted devices. While present designs, generally perform as intended in shorter-term applications, the major limitation of our current technology is inconsistent performance in longer term applications, which presently limits clinical implementation of this promising technology.

As early as 1974, chronic recording instability was noted following cortical implantation of recording electrodes [49]. Since then a number of studies have drawn attention to performance issues which arise with chronic recording electrode arrays, where typically the number of functional recording sites and the quality of signals observed diminish over time [29, 30, 50-52, 86]. Although the brain tissue response to implanted electrode arrays is believed to be a major contributing factor, the precise mechanisms that cause inconsistent recording performance are unknown. Accordingly, multiple hypotheses have been generated to describe how the tissue response contributes to the variability of chronic recording quality and longevity. Early hypotheses focused on the foreign body reaction as a walling off mechanism and a cause of device migration over time [49, 50]. Later hypotheses identified the astrocyte reaction and its potential to form a high impedance layer [94, 123, 130], or as a means to push neurons out of the recording zone [52]. More recently, the persistence of inflammation and neuronal cell death has been suggested as another mechanism that may affect reliability [95, 131].

A number of studies have examined the chronic brain tissue response to planar “Michigan style” silicon microelectrode arrays. While these studies have been informative it is not clear to what extent the observed reaction is a function of the composition of the device surface. To address this issue, we conformally coated planar Michigan SiMEAs with Parylene-C and compared the brain tissue reaction to uncoated controls following implantation into the cerebral cortex of adult male Sprague-Dawley rats at 2, 4, and 12 weeks postimplantation. Parylene-C is a common insulating coating for cortically implanted electrodes [50, 51, 57, 92, 133, 200], which has been shown to reduce inflammatory cell attachment *in vitro* [135]. Following implantation, animals

were sacrificed and cell-specific markers and quantitative methods were used to compare the tissue response spatially and temporally between uncoated and Parylene-C coated SiMEAs.

## Methods

### *Quantification of cell attachment*

Primary brain derived microglial cells were isolated using a variation of methods described previously [135]. Cerebral cortices of postnatal day 1 Sprague Dawley rats were isolated and minced after removing the meningeal layer. The tissue was then digested with 1.33% Collagenase Type IV in DMEM/F12 at 37 °C for 20 min. Next, the solution was centrifuged, the supernatant was removed by aspiration, and 4 ml of Trypsin-EDTA was added at 37 °C for 20 min. The resulting solution was treated for 2 minutes with SBTI, followed by centrifugation, and aspiration of the supernatant. Growth media was then added with 1% DNase, and cells were allowed to grow to confluence at 37 °C/5% CO<sub>2</sub>. Upon reaching confluence, purified microglia were obtained by mechanically agitating the mixed cortical preparations for 18 hours on an orbital shaker at 175 rpm. The media was then collected and centrifuged. The cell pellet was resuspended in DMEM-FBS, triturated, and assayed for viability.

Primary rat-derived microglial cells in solution were then seeded onto sterilized glass and Parylene-C-coated coverslips (diameter = 12 mm) at a volume of 0.5 ml and a density of 120,000 cells/ml. Twenty-four hours later, cells were fixed with 4% paraformaldehyde in PBS and processed for immunohistochemistry. In order to quantify the number of attached microglia, cells were treated with antibodies against OX-42



(CD11b, 0.5  $\mu\text{g/ml}$ , BD Biosciences, San Jose, CA) and CD68 (ED-1, 0.5  $\mu\text{g/ml}$ , AbD Serotec, Raleigh, NC). The total number of cells that were immunoreactive for both CD68 and OX-42 were counted on each coverslip, with  $n = 3\text{-}4$  per group.

### *Microelectrodes*

Nonworking single shank microelectrode arrays were provided by the Center for Neural Communication Technology (CNCT) at the University of Michigan. Microelectrodes were 5 mm in total length, with a width of 200  $\mu\text{m}$  at the base tapering down to 33  $\mu\text{m}$  at the tip, and a thickness of 15  $\mu\text{m}$  at the shank tapering down to 2  $\mu\text{m}$  at the tip (Figure 3-1 B). For handling, microelectrodes were attached to a 0.25 mm diameter stainless steel wire with UV-curable glue (MD-1181-M, Dymax, Torrington, CT) at the bond pads prior to sterilization. All electrodes were cleaned by immersion in 70% ethanol and rinsed several times in sterile DI water, followed by sterilization with ethylene oxide (EtO). Following EtO treatment, electrodes were allowed to outgas for at least 48 hours prior to implantation.

### *Conformal Parylene-C coating*

A conformal coating of Parylene-C (Cookson Electronics Equipment, Providence, RI) was applied to cleaned samples using conventional chemical vapor deposition. Cleaned microelectrodes attached to stainless steel wires, as well as cleaned glass coverslips and cleaned silicon wafers, were placed separately into a deposition chamber, in which solid Parylene-C was vaporized at 150  $^{\circ}\text{C}$ , followed by pyrolysis at 670  $^{\circ}\text{C}$  to yield the gaseous Parylene-C monomer. When the deposition chamber was returned to room temperature, Parylene-C polymerized and adsorbed conformally to the surface of

the samples, with excess monomers removed by vacuum during deposition. The homogeneity of the Parylene-C coating was confirmed by imaging with SEM. Parylene-C coated electrodes and coverslips were then sterilized with ethylene oxide and allowed to outgas prior to implantation.

### *Surface characterization*

In order to characterize the surface coating, the coated silicon wafers were analyzed using FTIR spectroscopy. In order to model the surface exposure to the biological environment, additional samples were exposed to PBS at 37°C for 2, 4, and 12 weeks in an incubator, and were then analyzed using FTIR. The thickness of the coating was assayed by cutting a cross section of coated electrodes embedded in acrylic resin, with the thickness quantified using light microscopy. Coating homogeneity was assessed using SEM.

### *Animal surgery*

Methods used in this study were similar to those previously reported [201]. Briefly, all animal procedures were conducted in accordance with the University of Utah Institutional Animal Care and Use Committee (IACUC). Adult male Sprague Dawley rats (200-225 g, n = 9 per group) were anesthetized via an intraperitoneal injection of ketamine (65 mg/kg), xylazine (7.5 mg/kg), and acepromazine (0.5 mg/kg). Animals' heads were shaved, and disinfected, followed by transfer to a stereotaxic frame. A 3 mm diameter burr hole positioned +0.2 mm forward of bregma, and 3 mm lateral to bregma was cut via trephination, and the microelectrodes on stainless steel wires were implanted to a depth of 2 mm. The electrodes were fixed into place by gluing the attached stainless

steel wire to the custom-fabricated polyurethane grommet [127] that fit into the burr hole using a UV-curable medical grade adhesive (MD 1181-M, Dymax). After the adhesive cured, the scalp incision was closed and the animals were allowed to recover.

#### *Euthanasia and tissue preparation*

At 2, 4, and 12 weeks postimplantation animals were terminally anesthetized via an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (30 mg/kg), and perfused transcardially with PBS followed by 4% paraformaldehyde in PBS, at a flow rate of 50 ml/min. The electrodes were carefully retrieved with fine surgical scissors and microdissection forceps, followed by removal of the brain. Brains and electrodes were postfixed separately at 4°C with 4% paraformaldehyde in PBS overnight. Following postfixation, brains were serially sectioned in the axial plane ( $n = 6$ , each group) at 40  $\mu\text{m}$  thickness on a microtome, to a depth of 2.9 mm, spanning the cerebral cortex. The remaining implanted animals were serially sectioned in the coronal plane ( $n = 3$ , each group) both ventrally and dorsally from the electrode-tissue interface to a distance of 1 mm in each direction. An additional unimplanted cohort ( $n = 3$ , each timepoint) were sectioned horizontally at 40  $\mu\text{m}$  to a depth of 2.9 mm and were used as staining controls.

#### *Immunomarkers*

Three out of every four serial sections were batch-reacted with antibodies against GFAP (2.4  $\mu\text{g/ml}$ , DAKO, Carpinteria CA), which identifies for an intermediate filament specific to astrocytes, and the three consecutive sections were alternately treated with antibodies against CD68 (ED-1, 0.5  $\mu\text{g/ml}$ , AbD Serotec, Raleigh, NC) for activated macrophages/microglia, NF-160 (1.0  $\mu\text{g/ml}$ , Sigma-Aldrich, St. Louis, MO) for neuronal

processes, and NeuN (2.0  $\mu\text{g/ml}$ , Millipore, Billerica, MA) for neuronal nuclei. Each fourth section was left for additional immunomarkers, including MAP-2 (2.0  $\mu\text{g/ml}$ , Millipore, Billerica, MA) for dendrites, Myelin-Oligodendrocyte Specific Protein (MOSP, 2.5  $\mu\text{g/ml}$ , Millipore, Billerica, MA) for myelin and oligodendrocytes, NF-200 (2.0  $\mu\text{g/ml}$ , Sigma-Aldrich, St. Louis, MO) for axons, and rat IgG (2.0  $\mu\text{g/ml}$ , Southern Biotech, Birmingham, AL) to assess blood-brain-barrier (BBB) integrity. Antibodies were diluted in a blocking solution consisting of 4% (v/v) goat serum (Invitrogen, Carlsbad CA), 0.5% (v/v) Triton-X 100, and 0.1% (w/v) sodium azide. Tissue sections were batch treated for 1 hour in a blocking solution at room temperature, followed by incubation with primary antibodies overnight at 4°C. After three rinses in PBS at room temperature to remove excess antibody (1 hour/each rinse step), appropriate fluorescently-labeled secondary antibodies were applied in block for 1 hour at room temperature, followed by three washes in PBS (1 hour/each rinse step). All sections were also counterstained with 10  $\mu\text{M}$  DAPI for cell nuclei. Tissue sections were mounted on microscope slides, covered with Fluormount-G (Southern Biotech), and coverslipped. Implanted electrodes were investigated using SEM, and were immunostained for GFAP and CD68 in a similar manner as tissue. Immunohistochemistry also included nonimplanted control animals ( $n=3$ , each timepoint), and primary controls in which the primary antibodies were omitted.

#### *Quantitative intensity analysis*

Fluorescent images of tissue sections from Layers V and VI of the cortex, near the tip of the microelectrodes, were captured with a Coolsnap digital camera and a Nikon Eclipse E600 microscope, using identical exposure times and conditions which were

optimized for each immunomarker. Additional sections were imaged using an Olympus FV 1000 confocal microscope. Lightfield corrections and background subtraction using primary controls were performed prior to line profile analysis. The fluorescent intensity was quantified, averaged, and compared using a custom LabView-based image analysis program (National Instruments) that takes a linear array of line profiles originating from the implant site and extracts the fluorescent intensity as a function of distance from the implant site.

For each section, line profiles were extended through the section originating at the electrode-tissue interface, at an angle of 90° to the long axis of the electrode, and at a spacing of 2.5-3.0  $\mu\text{m}$  between each line profile. At each point along the lines, an anti-alias pixel extraction algorithm was used to derive the pixel intensity value [127]. A mean intensity profile was then calculated by averaging all of the line profiles per section. The average intensity for each immunomarker was then combined with all the sections for each timepoint, to obtain an average intensity profile for the cohort. In order to quantify the changes in neuronal nuclei, images of sections immunoreacted with antibodies against NeuN were overlaid with a 50 x 50  $\mu\text{m}$  grid, originating at the electrode-tissue interface, and the number of NeuN<sup>+</sup> and DAPI<sup>+</sup> nuclei in each grid were counted through  $\pm 500$   $\mu\text{m}$  of the section, perpendicular to the long axis of the electrode. The number of neuronal nuclei counted was then compared to the number of NeuN<sup>+</sup> nuclei in unimplanted control animals.

### *Statistical analysis*

The area under the curve of each average intensity profile for each immunomarker at 2, 4 and 12 weeks in the first 100  $\mu\text{m}$  adjacent to the electrode, corresponding to the

critical recording zone, was compared across timepoints using an ANOVA and a Tukey posthoc test. Areas under the curve were analyzed using an SPSS software package, with significance considered at  $p < 0.05$ . The area under the curve in the recording zone was also compared between uncoated and Parylene-C-coated SiMEAs at each timepoint using a Student's  $t$  test.

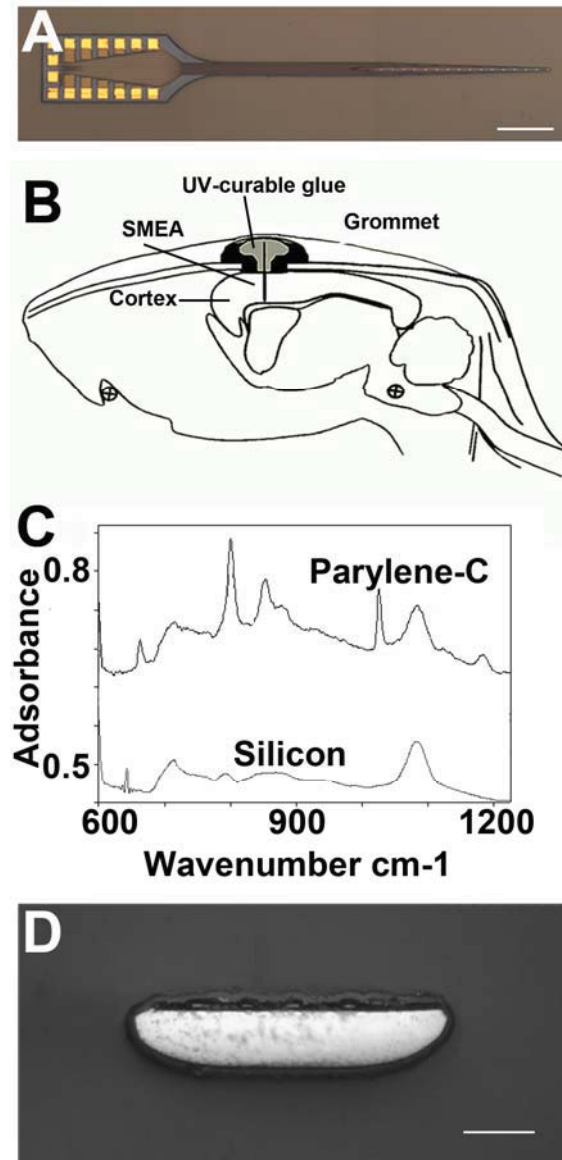
## Results

### *Surface analysis*

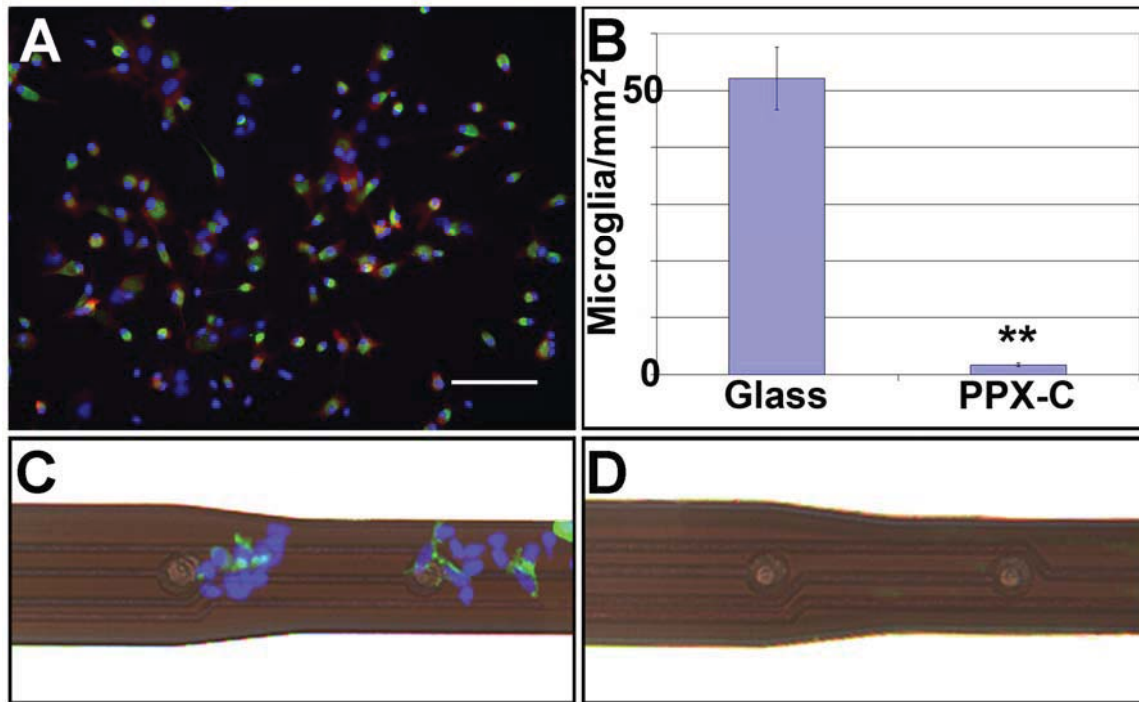
The coating was found to be conformal and homogeneous, as assessed by SEM (not shown), and the coating was found to be approximately 1  $\mu\text{m}$  thick, as shown in cross section in Figure 3-1 D. The identity of the coating was confirmed using FTIR, with the fingerprint region shown in Figure 3-1 C. In addition, the coating remained stable on the surface of the silicon wafer through 12 weeks of in vitro testing, as is shown in Figure 3-1 C.

### *Cell attachment to Parylene-C-coated and uncoated substrates*

Following seeding onto coverslips, cell attachment was quantified at 24 hours. Figure 3-2 E shows a representative view of attached microglial cells 24 hours after seeding on an uncoated coverslip. Many cells were observed attached to uncoated coverslips, but very few cells were observed attached to Parylene-C-coated coverslips. Figure 3-2 F shows the quantification of cell attachment 24 hours after cell seeding. There was a statistically significant 95% reduction in cell attachment to Parylene-C-coated coverslips ( $p < 0.001$ ).



**Figure 3-1.** Electrode coating. A) Single shank microelectrode with 16 recording sites. Scalebar = 500  $\mu\text{m}$ . B) CAD drawing of a mid sagittal section of rat head showing location of implant and anchoring polyurethane grommet. All microelectrodes were lowered stereotactically to a controlled depth of 2 mm below the surface of the cortex, and attached to a soft polyurethane grommet using a UV-curable adhesive as shown. C) FTIR spectra of the footprint region, showing Parylene coating 2, 4, and 12 weeks following in vitro testing. D) Brightfield image of a representative cross-section of a Parylene-C coated electrode with a 1  $\mu\text{m}$  coating of Parylene-C. Scalebar = 10  $\mu\text{m}$ .



**Figure 3-2.** Reduced cell attachment to Parylene-C-coated coverslips and SiMEAs. A). Representative image of cell attachment to an uncoated coverslip showing a high density of adherent activated microglia/ macrophages. Adherent microglia were immunoreacted with antibodies against CD68 (green) to assess activation, and antibodies against CD11b (OX-42, red) for morphology. B) Quantification of cell attachment on uncoated (glass) and Parylene-C-coated coverslips at 24 hours, showing a significant reduction in attached cells following Parylene-C coating of the coverslips. \*\*  $p \leq 0.001$ . Ex vivo results were similar. C). Representative fluorescent image of cell attachment to uncoated explanted SiMEA showing adherent activated microglia/ macrophages. Adherent cells were found along the length of the explanted electrode at each timepoint. D). Representative fluorescent image of cell attachment to same Parylene-C coated SiMEA showing no adherent activated microglia/ macrophages. Similar to in vitro results, at each timepoint explanted electrodes were found to be free from cellular attachment. All scalebars = 100  $\mu\text{m}$ .

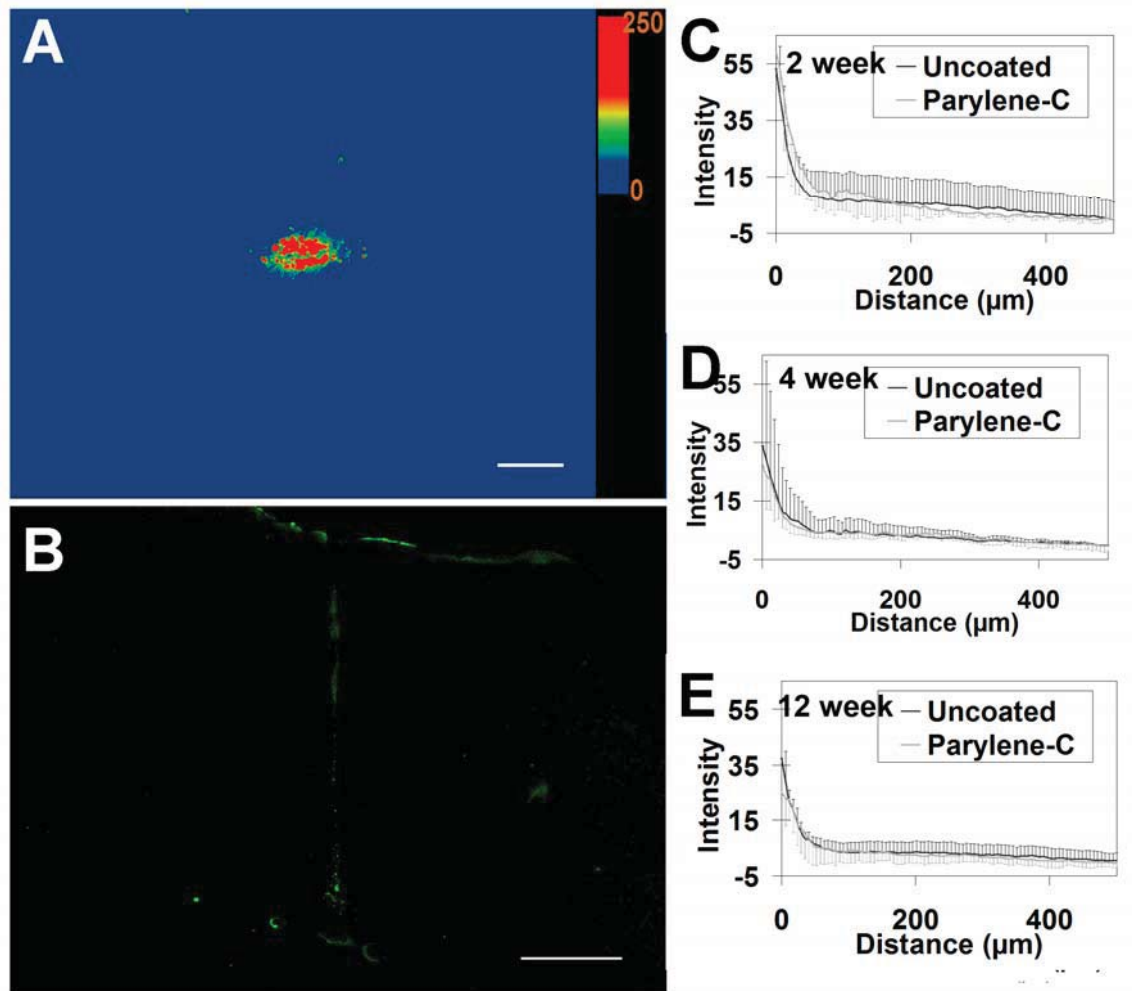


After being implanted for a duration of 2, 4, or 12 weeks ( $n = 9$ , each timepoint), cellular attachment was observed on uncoated SiMEAs at each timepoint, as is shown in Figure 3-2 B. However, similar to Parylene-C-coated coverslips, cellular attachment was not observed on Parylene-C-coated SiMEAs at any timepoint (Figure 3-2 D).

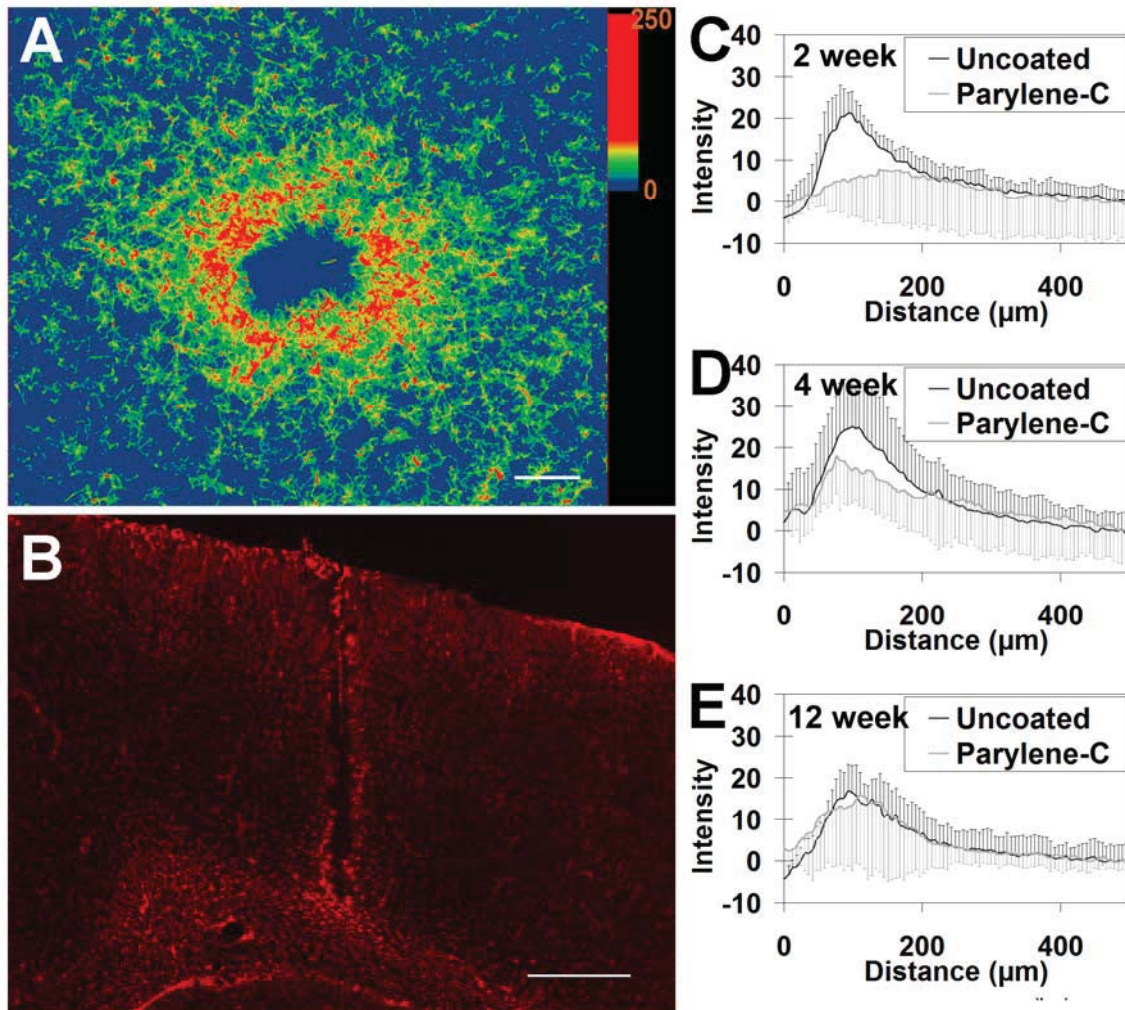
#### *Quantitative glial and neuronal response*

The lysosomal marker CD68 was used to identify microglia/ macrophage activation near the implant site. The CD68 response was similar to that which our group has described previously [95, 131, 135], and is shown in Figure 3-3. In general, CD68 immunoreactivity was only observed in the zone directly adjacent to the implant site, with immunoreactivity not extending more than 50  $\mu\text{m}$  from the electrode-tissue interface. Spatially, CD68 immunoreactivity was greatest along the broad face of the planar electrode, as opposed to the edges. No evidence of microglia/macrophage activation was observed in other brain areas, as is seen in the representative coronal section at 12 weeks. When the average profiles for uncoated and Parylene-C-coated SiMEAs were compared at each timepoint, no statistical differences were found. However, when the data were analyzed temporally, it was found that the CD68 immunoreactivity decreased between 2 and 12 weeks in both animal cohorts. In addition, the variability associated with CD68 immunoreactivity was found to decrease as a function of distance from the implant site and over time.

Figure 3-4 shows representative images and quantitative analysis of the GFAP immunoreactivity to uncoated and Parylene-C-coated SiMEAs. Astroglial encapsulation was not observed in any of the cohorts, but a layer of hypertrophic astrocytes was observed that peaked approximately 80 – 100  $\mu\text{m}$  from the implant track. Between this



**Figure 3-3.** CD68 immunoreactivity A) Average 12 week uncoated cohort surface plot showing CD68 immunoreactivity restricted to the region directly adjacent to the SiMEA. B) Representative coronal section from a SiMEA implant at 12 weeks showing the similarity of the reaction as a function of depth along the electrode. Average CD68  $\pm$  standard deviation as a function of distance from the interface C) 2 weeks, D) 4 weeks, and E) 12 weeks following implantation. Variability decreased both spatially and temporally The area under the curve of CD68 immunoreactivity in the recording zone did not change between animal cohorts implanted at any timepoint, but was found to decrease at 12 weeks ( $p < 0.05$ ) in both cohorts. Scalebar in A = 100  $\mu\text{m}$ , B = 500  $\mu\text{m}$ .



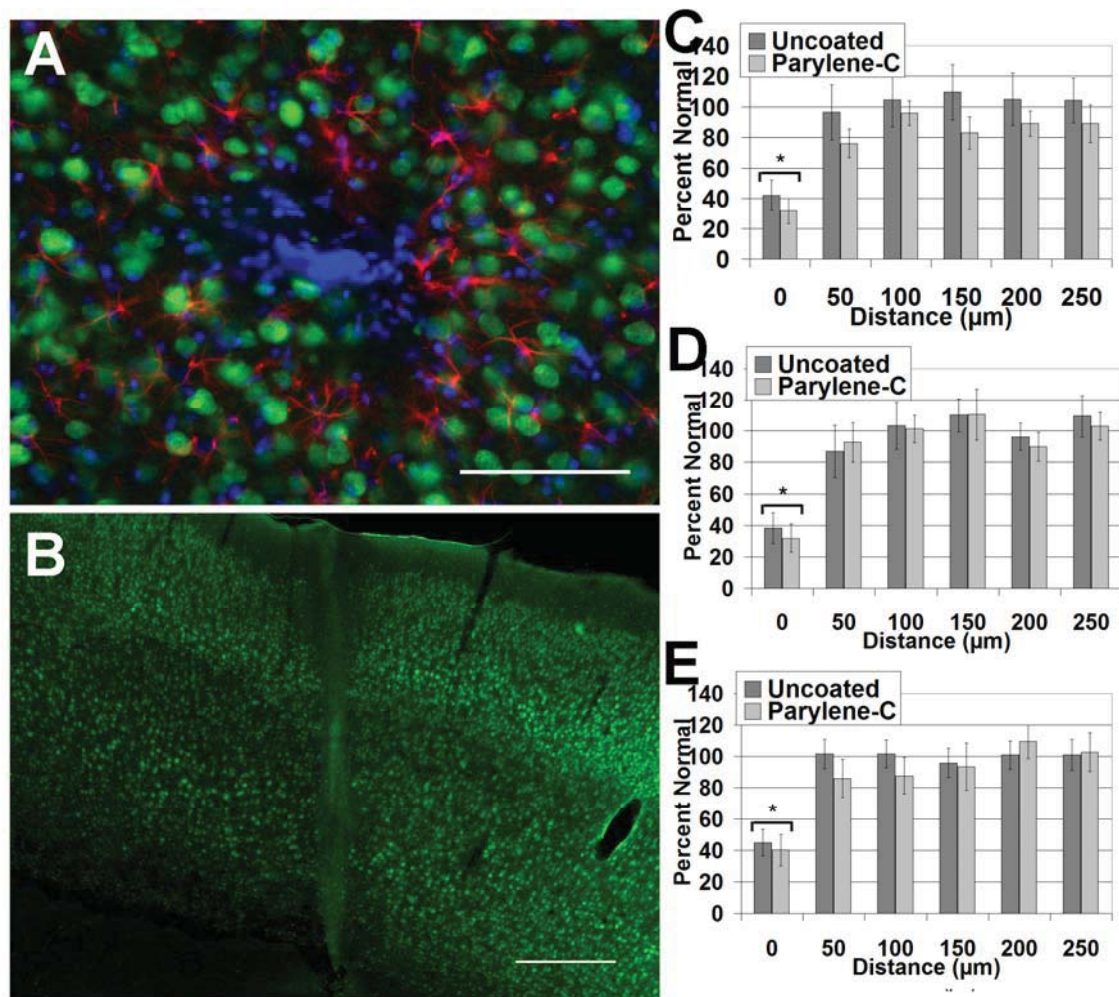
**Figure 3-4.** GFAP immunoreactivity. A) Average 12 week uncoated cohort surface plot showing GFAP immunoreactivity adjacent to the SiMEA. B) Representative coronal section from an uncoated implant at 12 weeks showing the similarity of the reaction as a function of distance along the long axis of the electrode. Note the lack of interdigitating GFAP<sup>+</sup> processes surrounding the implantation tract. Average GFAP as a function of distance from the interface  $\pm$  standard deviation C) 2 weeks, D) 4 weeks, and E) 12 weeks following implantation. The area under the curve of GFAP immunoreactivity in the recording zone (100  $\mu$ m) did not change between animal cohorts or temporally. Scalebar in A = 100  $\mu$ m, B = 500  $\mu$ m.

hypertrophic astrocyte layer and the SiMEAs, a layer that was not immunoreactive for GFAP was observed, but which stained extensively for DAPI. When the area under the curve of the recording zone from the quantitative fluorescence profiles was statistically analyzed, no differences were found between coated and uncoated SiMEAs at any timepoint. In addition, the average profiles were not found to change temporally.

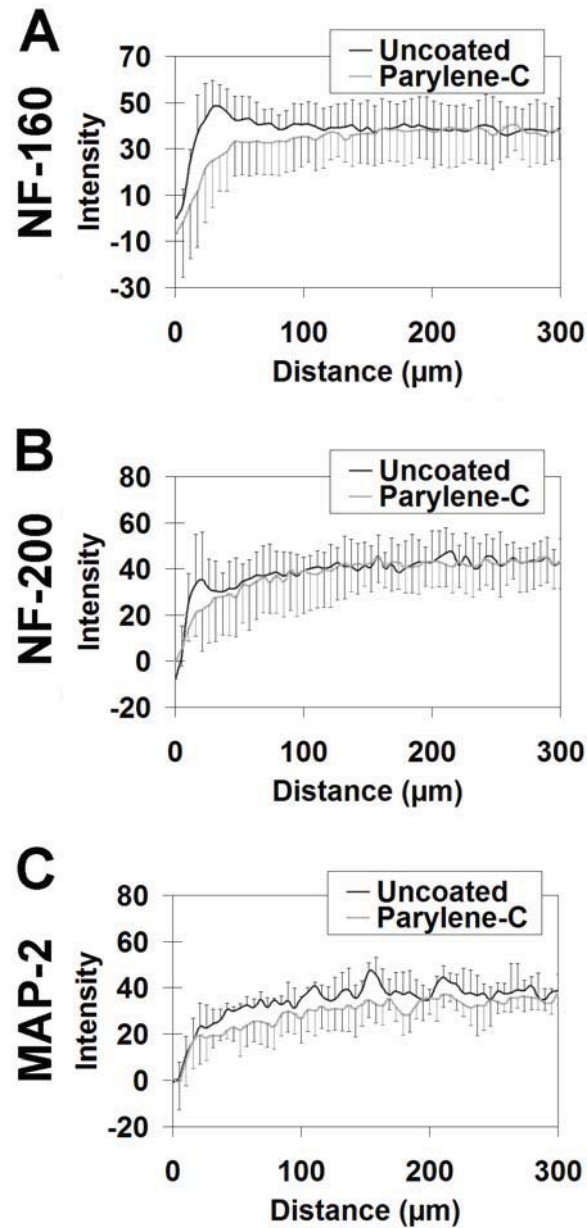
Neuronal nuclei were identified using antibodies against NeuN, and were quantified in discrete 50 x 50  $\mu\text{m}$  bins from the implant site by counting. The representative NeuN immunoreactive phenotypes for uncoated and Parylene-C-coated implants at each timepoint are shown in Figure 3-5. Within the first 50  $\mu\text{m}$  from the implant site an approximate 60% reduction in the number of neuronal nuclei was observed at each timepoint and with each implant type. Correspondingly, further than 50  $\mu\text{m}$  from the implant site the number of neuronal nuclei returned to a value that was not statistically significant from unimplanted control animals housed in the same facility for each cohort. No differences were found between uncoated and Parylene-C coated cohorts at any timepoint. In addition, when the cohorts were analyzed over time from 2 to 12 weeks, no differences were found in the reactive phenotype for this immunomarker.

Figure 3-6 shows the reaction of several neuron-specific cytoskeletal markers adjacent to both implants at the 12 week timepoint. At the 12 week timepoint, a regenerative phenotype was observed, with an increase in NF-160 immunoreactivity directly adjacent to the implant site for both the uncoated and Parylene-C coated cohorts (Figure 3-6 A). NF-200 in our hands detects axons alone, and outside of the implant site no decrease in NF-200 immunoreactivity was observed, and there was no difference between the uncoated and Parylene-C coated cohort (Figure 3-6 B). MAP-2 is associated





**Figure 3-5.** NeuN immunoreactivity. A) Representative 12 week image showing NeuN (green) and GFAP (red) immunoreactivity adjacent to the SiMEA. B) Representative coronal section from an uncoated implant at 12 weeks showing the similarity of the reaction as a function of depth along the electrode. Average NeuN as a function of distance from the interface +/- standard deviation C) 2 weeks, D) 4 weeks, and E) 12 weeks following implantation. There was no significant difference in the number of NeuN+ nuclei between cohorts at any timepoint, with all sections showing the same phenotype of decreased neuronal nuclei within the first 50  $\mu\text{m}$  from the implant. No temporal changes were found. Scalebar in A = 100  $\mu\text{m}$ , B = 500  $\mu\text{m}$ .



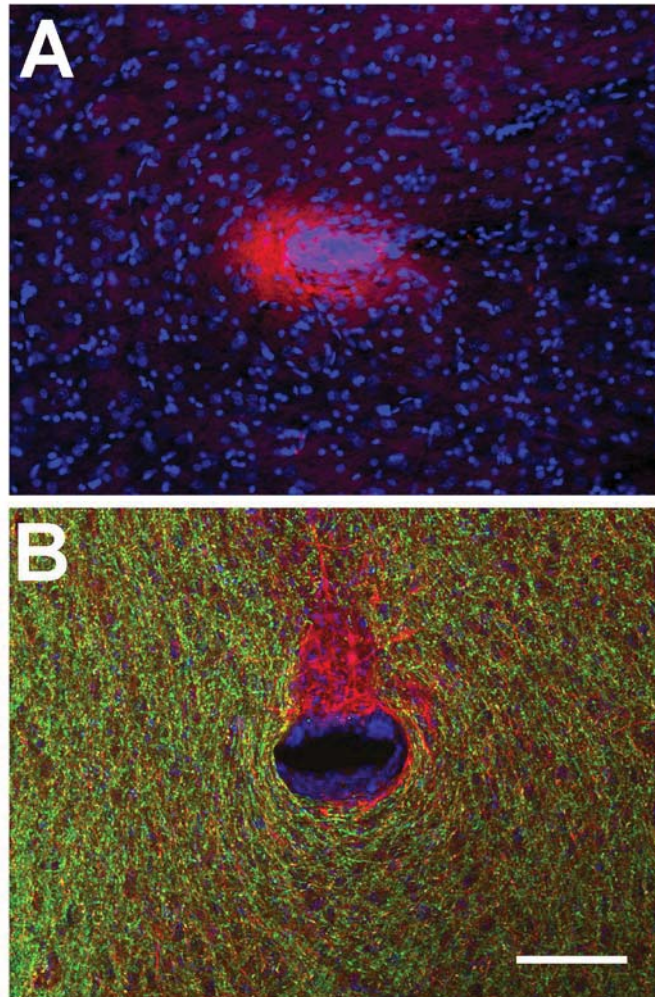
**Figure 3-6.** Neurofilament immunoreactivity at 12 weeks. The neuronal response in uncoated and Parylene-C-coated SiMEAs shown at 12 weeks following implantation immunoreactive for NF-160 (A), a marker of both axons and dendrites, NF-200 (B), a marker of axons, and MAP-2 (C), a marker for dendrites. In this later timepoint a more regenerative phenotype was observed. When the uncoated and Parylene-C-coated cohorts were compared for each immunomarker, no significant differences were found.

with microtubules in dendrites, and similar to the other neuronal markers, no changes outside the implant track were observed (Figures 3-6 C).

Additional sections were immunoreacted with antisera for IgG. In uninjured rat cortex, IgG is restricted to the vasculature, and is therefore used as an indicator of blood-brain barrier (BBB) integrity. As is shown in Figure 3-7 A, IgG immunoreactivity was observed in the region directly adjacent to the implant site irrespective of electrode coating, indicative of BBB dysfunction. Figure 3-7 B shows a representative section at 12 weeks immunoreactive for MOSP and NF-200. The tissue region directly adjacent to the implant site showed a decrease in myelin/oligodendrocyte immunoreactivity, irrespective of electrode coating, indicating myelin loss with axonal preservation.

### Discussion

In this study, we have shown that differences in the surface chemistry of implanted microelectrodes did not influence the foreign body response, suggesting that the stimulus causing the tissue response involves another device attribute. This suggests that other factors, such as electrode size, surface area, mechanical properties or device permeability have a stronger influence on the brain tissue reaction to chronically implanted microelectrode arrays. Our results show that a conformal coating of Parylene-C causes a 95% reduction in inflammatory cell attachment *in vitro* on microglia-seeded coverslips, as well as a nearly complete reduction *in vivo* on explanted SiMEAs. However, this coating did not affect the observed neuronal and glial reaction from 2 to 12 weeks. Indeed, the tissue reaction to silicon microelectrodes has been shown to be due to the presence of the implant, not the initial injury [95], and shares the same basic features



**Figure 3-7.** IgG and Myelin-Oligodendrocyte Specific Protein immunoreactivity. A. Representative horizontal section illustrating IgG immunoreactivity (red), which was observed principally surrounding the electrode tract, indicating leakiness of the BBB. B. Representative confocal image of a horizontal section showing immunoreactivity for MOSP (green) and NF-200 (red) illustrating a reduction of myelin-oligodendrocyte specific protein immunoreactivity directly adjacent to the implant track, in a zone with normal or slightly increased immunoreactivity for axons. DAPI is shown in blue in each panel. Scalebar = 100  $\mu$ m.



as the response to impermeable Pt-Ir DBS electrodes [148-151] and impermeable silastic CSF shunts [147] suggesting that the tissue response to CNS implants may be a general feature, regardless of implant surface chemistry.

One hallmark of the brain tissue reaction in various species using many different electrode designs is the presence of persistent, activated microglia at the implant site [94, 95, 105, 119, 121, 126, 129, 131, 134]. In the current study, we observed a similar reactive phenotype which was found to decrease over time from 2 to 12 weeks in both the uncoated and Parylene-C-coated cohorts. In the case of Parylene-C coated microelectrodes, microglia may have been activated without actually binding to the implant. If microglial attachment to surfaces were necessary for activation, we would have expected more devastating neuronal loss adjacent to uncoated SiMEAs as compared to Parylene-C-coated SiMEAs. In addition, the variability of the activated microglia/macrophage immunoreactivity was found to decrease over time, which may be due to a decrease in macrophage trafficking from the vasculature and periphery at later timepoints.

We found no changes in the immunoreactivity for GFAP in the recording zone temporally. GFAP immunoreactivity was increased in the first 150  $\mu\text{m}$  adjacent to each electrode at each timepoint, indicative of astrocyte hypertrophy. Various groups have focused on the astrocyte reaction as the major contributor to chronic recording failure, hypothesizing that a progressive astrocyte response walls off the electrode [94, 123, 130], or pushes neurons away from the recording zone [52]. Our evidence indicates that from 2 weeks to 12 weeks, neither the astrocyte reaction nor neuronal reduction increases, indicating that the tissue response does not push neurons away from the recording zone

over time. In addition, we observed some astrocyte hypertrophy but not dense glial scar formation, suggesting that in this application, astrocytes probably would not cause recording failure.

Consistent with previous quantitative reports of neuronal viability surrounding implants, the majority of the reduction in neuronal immunoreactivity we observed was within the first 50  $\mu\text{m}$  adjacent to the implant site [93, 95, 131, 133]. This degree of neuronal reduction should still support recording ability, since available evidence suggests that recording electrodes must be within a zone of approximately 50-100  $\mu\text{m}$  from viable neurons to record electrical activity in the cortex [47, 202, 203]. In a recent report by Zhong et al., neuronal loss adjacent to Dexamethasone-coated SiMEAs in the rat brain increased over the course of 4 weeks, while immunoreactivity for activated microglia decreased [134]. However, using a large cohort of animals and quantitative methods, our data and previous data [106] show no evidence for progressive neuronal loss. Conversely, our immunoreactivity for neurofilament-160 showed an increase in immunoreactivity directly adjacent to the implanted SiMEA at the 12 week timepoint, which was not observed with dendritic markers, and was more evident at later timepoints, indicative of axonal regeneration.

Evidence of axonal regeneration adjacent to chronically implanted microelectrodes has been reported previously [94, 105]. More recent studies have shown that axonal regeneration occurs following injuries which cause neuronal death by apoptosis, both in the cerebral cortex and in the hippocampus [192, 204-207]. In response to penetrating cortical injuries, the intensity of tissue damage has been shown to correlate both with the extent of microglial activation and with a reduction in

regeneration [171, 174, 175]. Therefore, with the small magnitude of activated microglia and macrophages we observed surrounding both the uncoated and Parylene-C-coated SiMEAs, the local cortical microenvironment may have become more permissive to limited regeneration.

Myelin reduction and oligodendrocyte dysfunction is a hallmark of neurological diseases such as multiple sclerosis and progressive multifocal leukoencephalopathy, and is often observed in injury models including electrode implantation [105, 112]. The reduction of myelin and oligodendrocyte immunoreactivity near the biotic-abiotic interface observed in our study may affect neuronal signaling and synaptic stability, which could result in the common observation of chronic cortical recording instability. In addition, since autologous IgG is not found in brain parenchyma under normal conditions [193, 194] but has been observed in the brain following damage to the BBB [195] our observations indicate a state of chronic damage to BBB integrity. This has the potential of causing changes in the extracellular ionic milieu, which could also account for chronic recording inconsistency, since neuronal silencing has been observed following loss of ionic gradients [208].

## CHAPTER 4

### CNS DEVICE-ASSOCIATED INFLAMMATION IS SUFFICIENT TO IMPAIR HIPPOCAMPAL NEUROGENESIS

#### Introduction

Neurogenesis is the process of generating functionally integrated neurons from neural progenitor and stem cells, and occurs throughout life in discrete brain regions including the dentate gyrus of the hippocampus and the subventricular zone [152, 153]. Adult neurogenesis is believed to be associated with learning and memory formation [154-156], as well as recovery following injury or disease [157-159]. Recent reports described decreases in adult neurogenesis following exposure to glucocorticoids [163], sleep deprivation [164], stress [165], and as a function of age [166, 167]. Positive regulation of neurogenesis has been observed in other experiments following exercise [168], environmental enrichment [169], learning [170], periods of hypoxia [171, 172], as well as seizure induction [173].

Inflammation has also been shown to strongly affect neurogenesis [209, 210]. Using bacterial endotoxin (LPS) injections, which result in a large degree of hippocampal inflammation, the number of newborn neurons has been shown to have a strong negative correlation with activation of microglia in the same zone [174]. This decrease in neurogenesis is believed to be caused by a spike in the local concentrations of cytokines

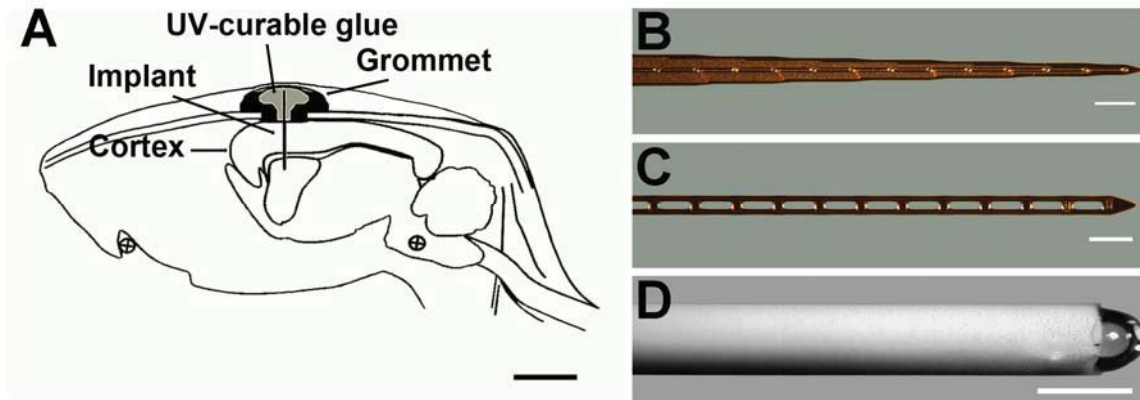
released by activated microglia, including TNF- $\alpha$  and IL-6 [175]. Not only does inflammation reduce hippocampal neurogenesis, but there is evidence to suggest it also alters functional connectivity in existing neuronal circuits [211]. Chronic inflammation is also associated with all known CNS devices during the indwelling period, including microelectrodes [95, 212], DBS electrodes [148-151], and hydrocephalic shunts [147]. However, whether inflammation associated with device implantation in the CNS is sufficient to cause changes to neurogenesis has not yet been reported.

In the current study, we asked whether the implantation of model recording microelectrodes and hollow fiber membranes (HFMs) into the brain of adult rats caused changes to hippocampal proliferation and the number of immature neurons 12 weeks following implantation. We used quantitative methods and image analysis to describe the inflammation associated with these implants, as well as changes to immunomarkers of neurogenesis.

## Methods

### *Microelectrodes*

Nonworking single shank planar microelectrode arrays were provided by the Center for Neural Communication Technology (CNCT) at the University of Michigan. Microelectrodes were 5 mm in total length, with a width of 200  $\mu\text{m}$  at the base tapering down to 33  $\mu\text{m}$  at the tip, and a thickness of 15  $\mu\text{m}$  at the base tapering down to 2  $\mu\text{m}$  at the tip (Experiments I and II; see Figure 4-1B). Custom planar microelectrodes with reduced surface area were provided by the Center for Wireless Integrated Microsystems at the University of Michigan. These microelectrodes had a 40  $\mu\text{m}$  x 10  $\mu\text{m}$  shank with a



**Figure 4-1.** Implantation diagram. A). Planar and lattice microelectrodes were stereotactically implanted to a depth of 3 mm from the top of the cortex (Experiment I) or 2 mm (Experiment II). HFM devices were implanted to a depth of 7.3 mm (Experiment III). Implants were then attached to the custom polyurethane grommet with a UV-curable glue. Scalebar = 5 mm. B). Planar silicon implants were 5 mm in total length, with a width of 200  $\mu\text{m}$  at the base tapering down to 33  $\mu\text{m}$  at the tip, and a thickness of 15  $\mu\text{m}$  at the base tapering down to 2  $\mu\text{m}$  at the tip. Scalebar = 100  $\mu\text{m}$ . C). Lattice silicon implants had a 40  $\mu\text{m}$  x 10  $\mu\text{m}$  shank with a total length of 3 mm, and a lattice structure incorporated within the shank, which reduces overall surface area by approximately 30%. Scalebar = 100  $\mu\text{m}$ . D). HFM implants had a total length of 7.5 mm and an outer diameter of 0.75 mm. The thickness of the outer skin of the HFM was approximately 60  $\mu\text{m}$ . Scalebar = 500  $\mu\text{m}$ .

total length of 3 mm. There was also a lattice structure incorporated within the shank, which reduces overall surface area by approximately 30% (Experiment I; see Figure 4-1 C). All microelectrodes were attached to a 0.25 mm diameter stainless steel wire with a UV-curable glue (MD-1181-M, Dymax, Torrington, CT) at the bond pads prior to sterilization. Electrodes were cleaned by immersion in 70% ethanol and rinsed several times in sterile DI water, followed by sterilization with ethylene oxide (EtO), and were allowed to outgas for at least 48 hours prior to implantation.

#### *Hollow fiber membrane implants*

Hollow fiber membrane devices were prepared as described previously [127]. HFMs had a total length of 7.5 mm and an outer diameter of 0.75 mm (Figure 4-1 D). The thickness of the outer skin of the HFM was approximately 60  $\mu\text{m}$ . Prior to implantation, HFM devices were sterilized by ultra-filtration with 70% ethanol and sterile DI water, and stored in sterile PBS until the time of implantation.

#### *Animal surgery*

All animal procedures were conducted in accordance with the University of Utah Institutional Animal Care and Use Committee. For each experiment, adult male Sprague Dawley rats (200-225 g,  $n = 5 - 6$  per group) were anesthetized via an intraperitoneal injection of ketamine (65 mg/kg), xylazine (7.5 mg/kg), and acepromazine (0.5 mg/kg). After reaching a full level of anesthesia as assessed by tail/toe pinch, the animals' heads were shaved, and the scalp was disinfected with 70% isopropanol and betadyne. Animals were then transferred to a stereotaxic frame, and a midline incision extending the length of the skull was made. The skin was retracted and the fascia was removed with a scalpel

blade and then dried with cotton tipped applicators. For experiment I, a 3 mm diameter burr hole was cut through the skull by a pneumatically driven trephine under PBS irrigation, with the center of the hole positioned -3.2 mm from bregma, and 2 mm lateral to bregma. For experiments II and III, the burr hole was cut at +0.2 mm forward of bregma, and 3 mm lateral to bregma. Using a stereomicroscope, the exposed dura was then opened with a 21 G needle. Microelectrodes and HFM devices were implanted as shown schematically in Figure 4-1 A. A custom fabricated polyurethane grommet was placed in the center of the burr hole. The microelectrodes were then lowered with a manual fine tooth stereotactic drive through the open lumen of the grommet to a depth of 3 mm from the surface of the cortex. Following implantation, the electrodes were fixed into place by gluing the attached stainless steel wire to a custom-fabricated polyurethane grommet [127] that fit into the burr hole using a UV-curable medical grade adhesive (MD 1181-M, Dymax). After the adhesive cured, the scalp incision was closed with 5/0 silk sutures and the animals were allowed to recover. (See Figure 4-1.)

#### *Euthanasia and tissue preparation*

At 12 weeks postimplantation animals were terminally anesthetized via an intraperitoneal injection of ketamine (70 mg/kg) and xylazine (30 mg/kg), and perfused transcardially with PBS followed by 4% paraformaldehyde in PBS, at a flow rate of 50 ml/min. The implants were carefully retrieved with fine surgical scissors and microdissection forceps, followed by removal of the brain. Brains and electrodes were postfixed separately at 4°C with 4% paraformaldehyde in PBS overnight. Following postfixation, brains were serially sectioned in the axial plane (n = 5 - 6, each group) at 40  $\mu$ m thickness on a microtome, to a depth of 8.5 mm, spanning the cerebral cortex and the

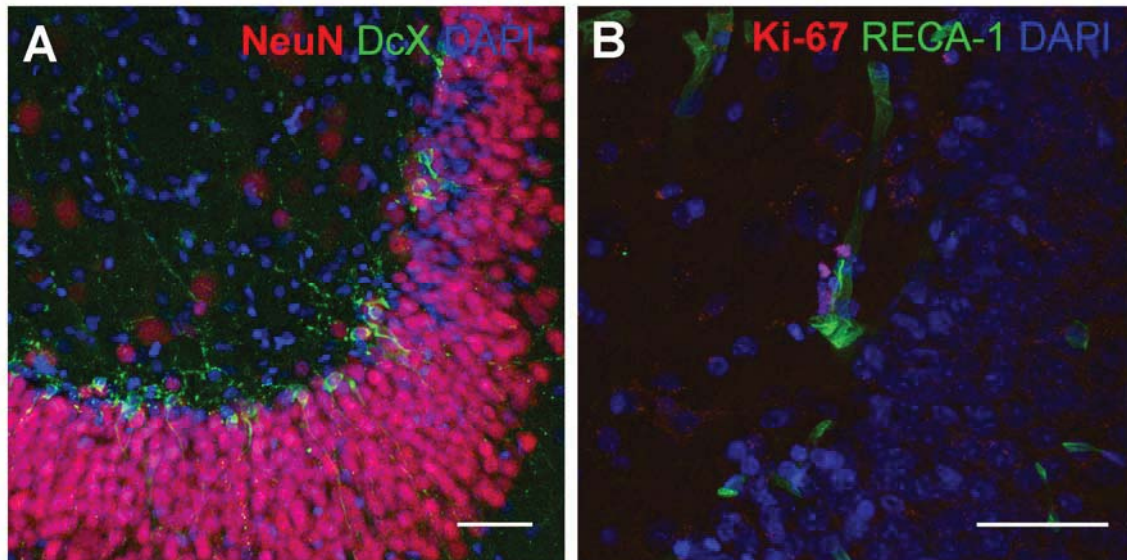


dentate gyrus of the hippocampus. An additional unimplanted cohort, housed for 12 weeks individually in the same facility ( $n = 6$ , each timepoint) were sectioned horizontally at 40  $\mu\text{m}$  in a similar manner as implanted animals.

### *Immunohistochemistry*

Sections from the cerebral cortex, corpus callosum, and hippocampus were batch-treated with GFAP (2.4  $\mu\text{g/ml}$ , DAKO, Carpinteria CA), for an intermediate filament specific to astrocytes, CD68 (ED-1, 0.5  $\mu\text{g/ml}$ , AbD Serotec, Raleigh, NC) for activated macrophages /microglia, NF-200 (2.0  $\mu\text{g/ml}$ , Sigma-Aldrich, St. Louis, MO) for axonal processes, MAP-2 (2.0  $\mu\text{g/ml}$ , Millipore, Billerica, MA) for dendrites, NeuN (2.0  $\mu\text{g/ml}$ , Millipore, Billerica, MA) for neuronal nuclei, rat IgG (2.0  $\mu\text{g/ml}$ , Southern Biotech, Birmingham, AL) to assess blood-brain-barrier (BBB) integrity, and Nestin (5.1  $\mu\text{g/ml}$ , Millipore, Billerica, MA) for neural precursor cells. Antibodies were diluted in a blocking solution consisting of 4% (v/v) goat serum (Invitrogen, Carlsbad CA), 0.5% (v/v) Triton-X 100, and 0.1% (w/v) sodium azide.

Every 10<sup>th</sup> serial section, encompassing the entire dentate gyrus (11 sections per animal) was treated with antibodies against Ki-67 (0.4  $\mu\text{g/ml}$ , Novocastra, Newcastle Upon Tyne, U.K.) for the nuclei of proliferative cells, and doublecortin (DcX, 10  $\mu\text{g/ml}$ , Santa Cruz Biotechnology, Santa Cruz, CA) for immature neurons (Figure 4-2). These antibodies were diluted in blocking solution consisting of 4% (v/v) goat serum for Ki-67 (Invitrogen, Carlsbad CA), or 4% (v/v) donkey serum for DcX (Millipore, Billerica, MA), and 1.0% (v/v) Triton-X 100 with 0.1% (w/v) sodium azide. All tissue sections were batch treated for 1 hour in a blocking solution at room temperature, followed by incubation with primary antibodies for 48 hours at 4°C. After three rinses in PBS at room



**Figure 4-2.** DcX and Ki-67. A). Representative horizontal section from the DG showing DcX immunoreactivity, which was restricted to the inner layer of the dentate gyrus, with mature NeuN<sup>+</sup> neuronal nuclei making up the bulk of the DG. B). Representative horizontal section illustrating Ki-67<sup>+</sup> proliferative nuclei, which were also generally observed in the inner layer of the DG, adjacent to RECA-1<sup>+</sup> vasculature. Scale bars = 50  $\mu$ m.

temperature to remove excess antibody (1 hour/each rinse step), appropriate fluorescently-labeled secondary antibodies were applied in block for 1 hour at room temperature, followed by three washes in PBS (1 hour/each rinse step). All sections were also counterstained with 10  $\mu$ M DAPI for cell nuclei. Tissue sections were mounted on microscope slides, covered with Fluormount-G (Southern Biotech), and coverslipped. Immunohistochemical processing also included nonimplanted control animals (n=6, each timepoint), and primary controls in which the primary antibodies were omitted.

### *Quantification*

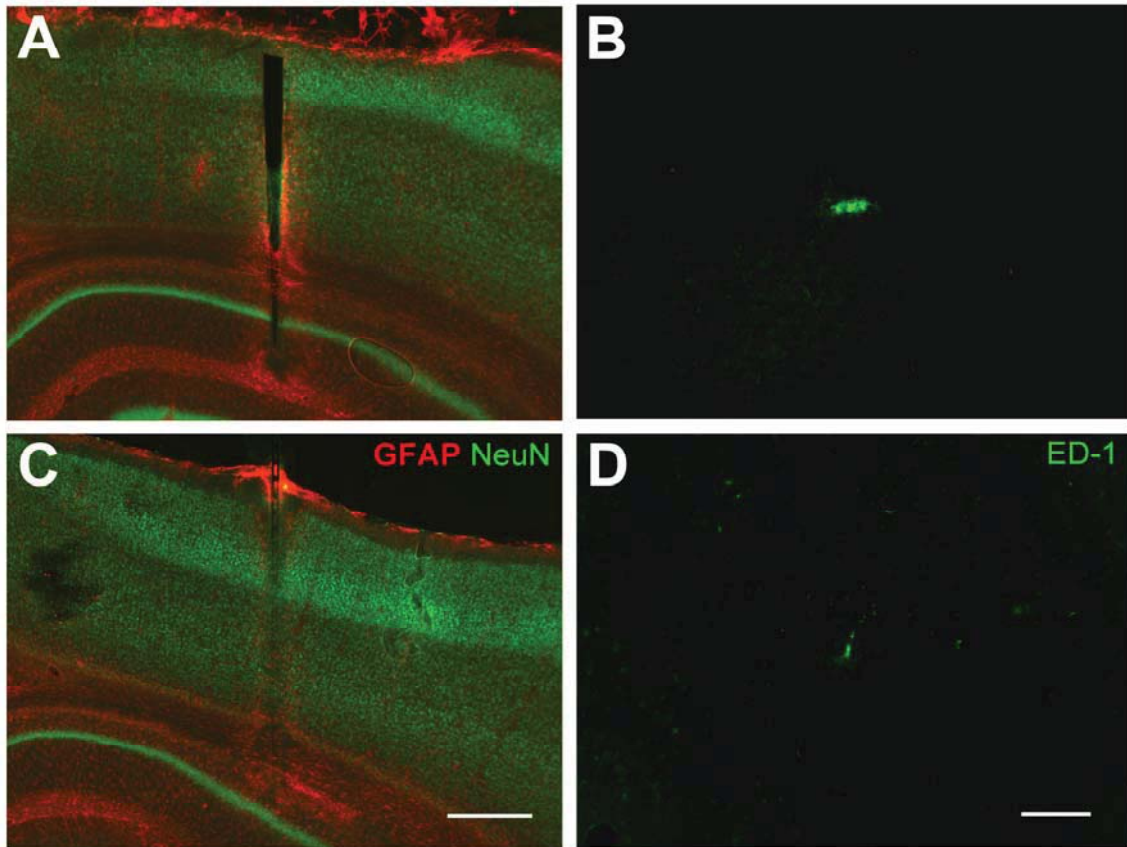
Sections from the dentate gyrus were imaged at a total magnification of 200x using a computer-controlled x-y stage which takes a series of images with ~10% overlap from the previous image. Following imaging, high quality montages of the entire dentate gyrus are created using the photomontage function in Adobe Photoshop. The number of Ki-67 and DcX positive cells in the granule cell layer (gcl) and the sub granule zone (sgz) of the dentate gyrus, as shown in Figure 4-2, was determined using the optical fractionator method [213, 214] by counting exhaustively all positive cells within a section. In order to detect differences between experimental groups, age-matched nonimplanted control animals were included at each timepoint, and the implanted (ipsilateral) dentate gyrus was counted separately from the unimplanted (contralateral) side to account for local inflammatory effects. The summed counts were then multiplied by 10 to report the number of immunopositive cells per DG.

### *Statistical analysis*

The average number of Ki-67<sup>+</sup> and DcX<sup>+</sup> cells per group was compared using ANOVA and a Tukey posthoc test with an SPSS software package, with significance considered at  $p < 0.05$ . The average number of Ki-67<sup>+</sup> and DcX<sup>+</sup> cells per group was also compared between the implanted and unimplanted dentate gyrus for each group using a Student's paired t-test.

## Results

Figure 4-3 summarizes the cortical tissue response at 12 weeks in both the planar and lattice implanted cohorts. Similar to previous descriptions at 12 weeks [135, 201], we observed microglia/macrophage activation at the biotic-abiotic interface, astrocyte

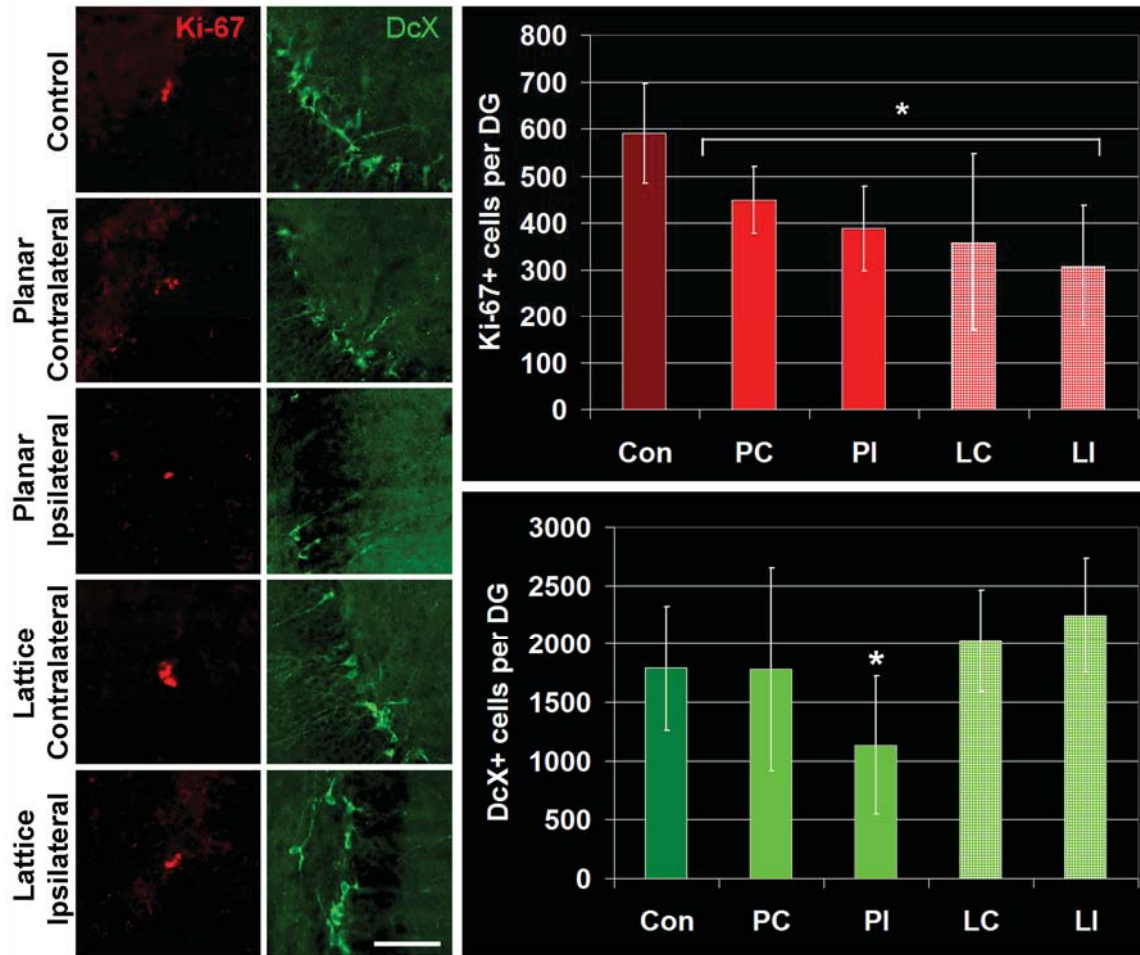


**Figure 4-3.** Planar and lattice tissue response. A) Representative 100  $\mu\text{m}$  coronal sections with electrodes captured within the section were used to assess the tissue response using NeuN and GFAP at 12 weeks. Both planar (A) and lattice (C) electrodes showed some neuronal loss and astrocyte hypertrophy in the cortex and CA1 of the hippocampus. The lattice electrode (D) showed reduced inflammation, as assessed by CD68 in horizontal sections, compared to planar electrodes (B). All scalebars = 100  $\mu\text{m}$ .

hypertrophy, and reduction in immunoreactivity for mature neurons (NeuN) adjacent to the electrode-tissue interface. CD68 immunoreactivity was increased adjacent to planar microelectrode implants as compared to the lattice microelectrodes, as was astrocyte hypertrophy as assessed by GFAP immunoreactivity. An interfacial reduction in NeuN<sup>+</sup> mature neurons was observed both in the cortex and CA1 of the hippocampus of both cohorts, but was more severe in the planar cohort.

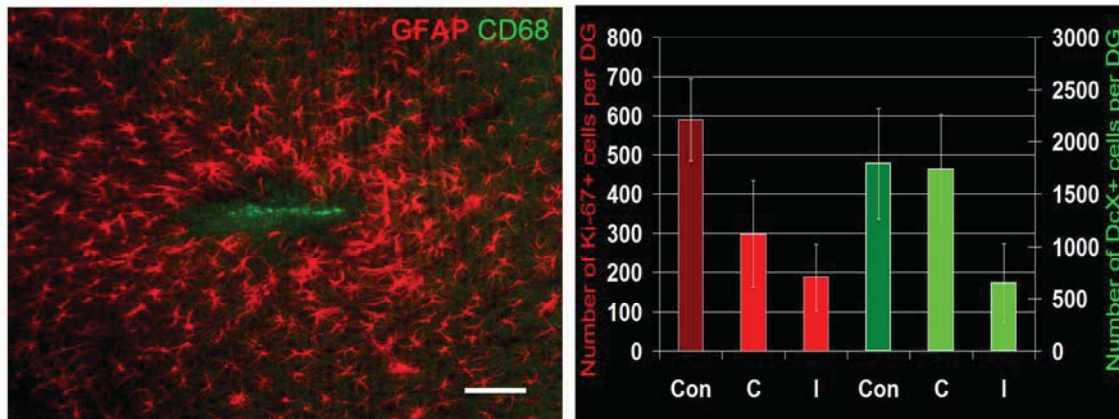
When the number of Ki-67<sup>+</sup> nuclei was quantified, a reduction in the number of proliferative nuclei was found in both the implanted and unimplanted hippocampi for both implanted cohorts as compared to age-matched controls housed in the same animal facility ( $p < 0.05$ ). The number of DcX<sup>+</sup> immature neurons was similarly quantified, and was found to be reduced only in the implanted hippocampus of the planar cohort ( $p < 0.05$ ), with no differences found in the unimplanted hippocampus and both hippocampi of the lattice cohort. Immunoreactivity for DcX and Ki-67 and quantification is summarized in Figure 4-4.

In order to understand whether the reduction in proliferation and the number of new neurons we observed in the planar cohort was due to the location of the microelectrode in the hippocampus, we implanted another cohort of animals with the same electrode, but restricted the implantation site to the cortex. As is shown in Figure 4-5, the tissue response in the cortex was similar to previous descriptions, including a local inflammatory reaction, assessed by CD68 immunoreactivity, astrocyte hypertrophy, and local neuronal loss (data not shown). When the number of proliferative cells and immature neurons in the dentate gyrus was quantified, a reduction in the number of Ki-67<sup>+</sup> nuclei was found in both the contralateral and ipsilateral DG, similar to hippocampal



**Figure 4-4.** Planar and lattice hippocampal neurogenesis quantification. Quantification of the number of proliferative cells, as assessed by Ki-67, and the number of immature neurons, as assessed by DcX, in the dentate gyrus of animals implanted with planar and lattice electrodes, as compared to age-matched, unimplanted controls housed in the same facility. A reduction in the number of proliferative cells was observed in both the ipsilateral and contralateral dentate gyrus of all implanted animals as compared to controls (\*  $p < 0.05$ ), but a reduction in the number of DcX+ cells was only observed in the ipsilateral dentate gyrus in the planar cohort (\*  $p < 0.05$ ). Shown are means  $\pm$  standard deviations. Scalebar =100  $\mu$ m. Con = control; P = planar; I=ipsilateral (implanted side).





**Figure 4-5.** Response adjacent to cortically implanted planar SiMEAs at 12 weeks. The cortical tissue response revealed CD68 immunoreactivity directly adjacent to the implant site, surrounded by a hypertrophic GFAP<sup>+</sup> immunoreactive zone. Quantification of number of proliferative cells showed a significant reduction in proliferation in both the ipsilateral and contralateral dentate gyrus as compared to control ( $p < 0.05$ ). Quantification of the number of DcX<sup>+</sup> immature neurons revealed a significant reduction in the ipsilateral dentate gyrus as compared to control ( $p < 0.001$ ). Shown are means  $\pm$  standard deviations. Scalebars = 100  $\mu$ m. Con = control; P = planar; I=ipsilateral (implanted side).

implants ( $p < 0.001$ ). In addition, the number of DcX<sup>+</sup> immature neurons was found to be reduced only in the ipsilateral DG ( $p < 0.001$ ).

In order to understand whether the reduction in proliferation and the number of immature neurons was restricted to devices made from silicon, we implanted another cohort of animals with a PAN-PVC HFM, to a depth of 7.3 mm, rostral to the hippocampus. The tissue response was similar to transcranial HFM implants described previously [127], and consisted of an inflammatory reaction characterized by CD68+

microglia/macrophages at the tissue interface, astrocyte hypertrophy, and neuronal loss, as shown in Figure 4-6. The number of Ki-67+ proliferative nuclei were found to be reduced in both the ipsilateral and contralateral DG ( $p < 0.05$ ), and the number of DcX+ immature neurons was found to be reduced only in the ipsilateral DG ( $p < 0.001$ ).

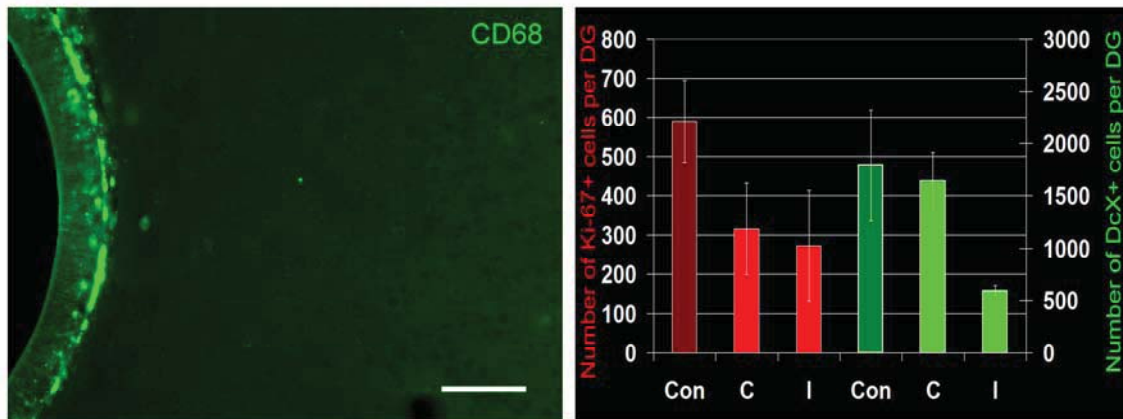
#### *Nestin immunoreactivity*

Additional sections were treated with antibodies against nestin, a type VI intermediate filament expressed in neural precursor cells. Nestin immunoreactivity was observed within both the vasculature [215, 216], as well as in neural precursors of the sgz, which were also GFAP+. A reduction in the number of nestin+/GFAP+ neural precursors was observed in the sgz of animals with implanted electrodes as compared to controls, but we did not have enough sections to fully quantify this trend (Figure 4-7).

### Discussion

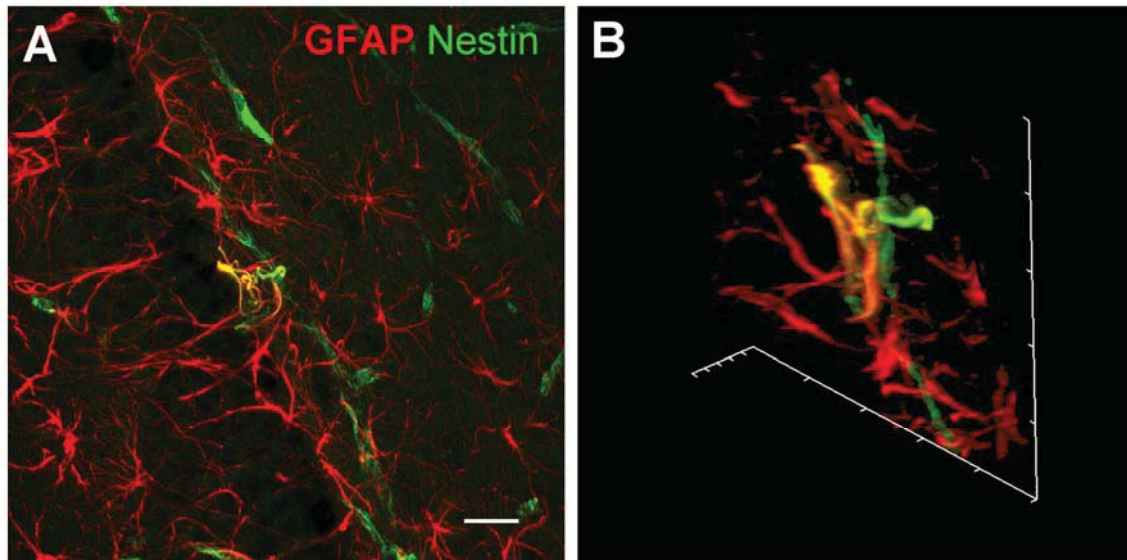
This study shows that the implantation of passive silicon microelectrodes and HFM devices does indeed reduce several stages of neurogenesis, including cellular proliferation, and the number of both neural precursors and immature neurons in the dentate gyrus on the implanted side of the brain. While the hippocampal proliferative response to CNS implants has not been previously reported, many reports have described changes in memory and intelligence following clinical hydrocephalic shunting and deep brain stimulator implantation. These reports in both children and adults have described reduced performance IQ and memory impairment following hydrocephalic shunting [176-178]. The implantation of stimulating electrodes has been used to treat symptoms associated with Parkinson's disease [20], depression [21], and is being investigated for





**Figure 4-6.** Response adjacent to implanted HFMs at 12 weeks. HFMs were implanted rostral to the hippocampus, and to a depth of 7.3 mm. Top left panel shows a representative horizontal section of the cortical tissue response adjacent to the HFM, including activated macrophage/microglia presence and glial hypertrophy. Quantification of number of proliferative cells showed a significant reduction in proliferation in both the ipsilateral and contralateral dentate gyrus as compared to control ( $p < 0.05$ ). Quantification of the number of immature neurons revealed a significant reduction in the ipsilateral dentate gyrus as compared to control ( $p < 0.001$ ). Shown are means  $\pm$  standard deviations. Scalebar = 100  $\mu$ m. Con = control; P = planar; I=ipsilateral (implanted side).

use in treating obsessive-compulsive disorder [22] and epilepsy [23]. While the use of DBS may relieve symptoms, evidence in the literature suggests that, like hydrocephalic shunting, DBS also causes cognitive impairments in patients following implantation [180-183], suggesting that decreases in memory and intelligence may be a general feature of penetrating brain implants, regardless of implant type, since inflammation is likely to persist throughout the lifetime of the implant [189]. Recently Hirshler et al. showed that



**Figure 4-7.** Nestin immunoreactivity. A. Representative horizontal section from the DG showing nestin immunoreactivity, either as a marker for the cerebral vasculature, or when associated with GFAP, marking the neural precursor cells of the sgz. B. Three-dimensional reconstruction of the GFAP+/nestin+ neuronal precursor cell from A. Note the close association with nestin+ vasculature. Scale bar = 20  $\mu$ m.

widespread neuroinflammation accompanies the implantation of DBS electrodes in the healthy rat brain which was also associated with memory deficits [217]. Here, we show a mechanism by which device factors cause memory deficits by reducing hippocampal neurogenesis.

Interestingly, the architecture of the implant affected the reaction. The surface area of the planar silicon electrode implanted to a depth of 3 mm was 0.69 mm<sup>2</sup> compared to the lattice implant of 0.30 mm<sup>2</sup>, with the lattice electrode being the only implant that did not significantly reduce the number of immature neurons in the implanted hippocampus. This suggests that there may be a cutoff of implanted surface area below which large changes to neurogenesis do not occur. Gelb et al. [218] showed using the rat

subcutaneous air pouch model that as the surface area of implanted PMMA particles increased, a threshold was reached at which point the inflammatory reaction increased dramatically. They hypothesized that the increased surface area resulted in increased protein adsorption, allowing enhanced interaction with immunocompetent cells. Our data agree with this hypothesis.

This study also shows that the location of the implant in the brain is not responsible for the changes to proliferation and the birth of new neurons in the dentate gyrus. A reduction in both metrics was seen with implants of similar surface area placed forward of bregma as well as those placed 3 mm caudal and closer to the midline. Similar inflammation was observed regardless of implant type or locations 12 weeks following surgery.

One assumption of our work is that the inflammatory response and changes to proliferation and neurogenesis were due to the chronic presence of the indwelling implant, and not the initial penetrating injury. Biran et al. [95] showed that the response to the initial injury of an electrode implant had disappeared by 4 weeks. However, the initial injury has been shown to affect cognition in a model of postoperative cognitive dysfunction in rats [219]. In addition, we did not address the inflammatory component the polyurethane grommet adds to the overall inflammatory response or hippocampal proliferation and neurogenesis. Herz et al. [179] showed in a rat model of cerebral recording using penetrating electrodes that the implantation of a headplug and cable, without invasion of the cranial cavity, was sufficient to cause cognitive dysfunction a week following implantation. Similarly, we believe that a portion of the reaction was due to the inflammation associated with the polyurethane grommet used in the craniotomy.

## CHAPTER 5

### SUMMARY, CONCLUSIONS, AND FUTURE WORK

#### Summary and Conclusions

As described previously, disease and injury to the CNS can be extremely debilitating to patients and costly to caregivers. With so many patients affected, several device-based therapies have become common in the clinic. One field that has much potential is using cortically implanted recording electrodes to provide therapies to patients affected by SCI and other movement disorders. However, chronic recording instability has limited clinical development of such devices, which is likely associated with the FBR to such silicon and metal-based electrode recording systems. As apparent in a review of the literature, microwires and microwire arrays have achieved the greatest success in terms of chronic recording, but the fundamental tissue response to such devices has not been characterized previously using quantitative techniques.

In Chapter 2, the quantitative tissue analysis to a large cohort of rats at both acute and chronic timepoints was shown following the implantation of a single microwire electrode. Previous hypotheses in the literature described progressive neuronal loss and dense astrocyte encapsulation as likely sources for reducing the ability of implanted electrodes to function reliably in chronic applications. We showed that neuronal loss does not progress in terms of both the mean number of neuronal nuclei and the density of

neuronal processes from 2 to 12 weeks. In addition, we showed not only that neuronal loss was restricted to the first 50  $\mu\text{m}$  adjacent to the implanted microwire, but also that neurofilaments tended to increase adjacent to the microwire over time, indicative of neuronal regeneration. We also showed that astrocytes did not encapsulate the microwire, even though astrocyte processes tended to hypertrophy, did not form a syncytium surrounding implants, and did not push neurons out of the recording zone over time, but contracted around the device between 2 and 4 weeks and remained stable thereafter. Another observation was CD68 immunoreactivity surrounding the implant at each timepoint investigated, indicative of chronic macrophage/microglial activation. Previously, chronic BBB damage and myelin loss has not been described adjacent to the current generation of penetrating microelectrodes. These observations prompted alternative hypotheses as to how the foreign body response may affect device function, either by modifying the extracellular ionic milieu in the case of an open BBB, or by causing cellular level electrical dysfunction by removing the myelin sheath.

In Chapter 3, the tissue response was quantitatively analyzed in planar silicon microelectrodes with a surface chemistry that had been drastically altered by a thin conformal coating of Parylene-C. The basic premise was to test how surface chemistry affected the chronic FBR. We observed the same general features of the FBR as had been described for microwires in Chapter 2, as well as for other penetrating brain implants including DBS electrodes and cerebrospinal shunts. This suggests that the FBR may be a general feature to penetrating implants, regardless of device attributes such as geometry and materiality. When we investigated whether Parylene-C changed the FBR, we found no difference between the cohorts implanted with uncoated SiMEAs and those

implanted with coated SiMEAs. This suggests that the surface chemistry of the implant plays only a minor role on the ensuing tissue reaction, and that other properties, including mechanical properties such as stiffness and surface area, or porosity may play a larger role in the FBR. Another point is that, similar to work with the microwire, we observed evidence of chronic microglia/macrophage activation adjacent to both the coated and uncoated cohorts, indicating that inflammation may persist over the lifetime of any implanted penetrating device.

In Chapter 4, we studied whether the inflammation that is always associated with penetrating devices in the brain was sufficient to impair neurogenesis in the hippocampus. With the negative correlation between inflammation and hippocampal neurogenesis well established in the literature for animal models of the inflammatory reaction caused by bacterial infection, we sought to define the relationship between the inflammation associated with device implantation and neurogenesis. To our knowledge, no other group has attempted to correlate device implantation and changes to neurogenesis. We found that implanted SiMEAs in the hippocampus did indeed reduce neurogenesis, as assessed by both the absolute number of new neurons in the dentate gyrus of the implanted hippocampus, as well as the number of proliferative cells in both hippocampi as compared to age-matched unimplanted controls. Next we asked whether this effect was due to the implant location, and found that implants in the cortex produced an identical effect on hippocampal neurogenesis. We also asked whether the response was due to the material implanted, and implanted a cohort of animals with polymeric PAN-PVC HFMs, which elicited a similar decrease to hippocampal neurogenesis. Finally, we sought to understand if changing device fabrication, by the addition of a

lattice structure to planar SiMEAs, which is expected to change both the implanted surface area of the device as well as device stiffness, would alter the effect on neurogenesis or the chronic FBR. Lattice electrodes, as compared to solid SiMEAs, were associated with less microglia/macrophage activation adjacent to the probe, which may be due to having reduced area for these inflammatory cells to bind, and secrete neurotoxic cytokines. In addition, even though we observed a bilateral reduction in the number of proliferative cells with all implants, we did not observe a reduction in the number of immature neurons in the cohort implanted with the lattice electrodes. This suggests that devices can be created with passive antagonism, in which anti-inflammatory strategies are manufactured into the device design without the need for pharmacological intervention.

### Future Work

As put forth in the preceding chapters, much is now known about the fundamental brain tissue response to penetrating implants. However, the signals necessary to control devices and computers in neuroprosthetic applications will require the use of multi-shank recording arrays in order to record from populations of neurons [220]. To date, there are no quantitative reports of the tissue response to multishank recording arrays. Reports of the tissue response to electrode arrays generally consist of single images, without sufficient information to determine the area from which the section was taken, whether the image shown is representative of the group, and the variability of the reaction in a single animal and within the group. The only group that attempted to quantify the tissue reactivity to an array was Edell et al [93]. Using a planar 10-shank silicon microelectrode

array, this group showed using histologically stained sections a statistically significant reduction in the number of neurons in the 50  $\mu\text{m}$  adjacent to individual shanks, which we have confirmed using single microwire implants and planar silicon microelectrode arrays. However, Edell et al. do not comment on the glial reaction or other characteristics of the tissue response.

It is likely that, since the activated microglia/macrophage response has been observed at every timepoint adjacent to implanted electrodes, and since the microglia/macrophage response affects a similar tissue region as that observed to have neuronal loss, the effects of close interwire spacing may be additive and produce a more severe tissue response. Recently this was proposed by Bridge et al. [221] using a 2-D finite difference model and published values of pro-inflammatory cytokine diffusivity. Assuming that the amount of activated microglia/macrophages was proportional to the amount of implanted surface, and that they serve as a chronic source of proinflammatory cytokines, Bridge showed that interelectrode spacing can significantly affect the level of cytokines adjacent to implants. Other reports have suggested that there is high variability of recording quality among individual electrodes of a multielectrode array [52, 76]. It is possible that the variability associated with chronic recording may be due to proximity to local vasculature, which may in turn affect local macrophage trafficking and subsequent inflammation, demyelination, and other reactive changes. In order to fully understand how the tissue response affects chronic recording quality, it will be necessary to quantitatively analyze markers of the tissue reaction to implanted arrays as a function of indwelling time, and compare them to recording quality using appropriate statistical methods.



Activated macrophages, as a hallmark of the foreign body response to biomaterial implants, remain at the tissue-biomaterial interface for the life of the implant (reviewed by [222, 223]). This was shown in previous chapters adjacent to implants of silicon probes, stainless steel microwires, and hollow fiber membranes implanted in the brain. However, we were not able to distinguish blood-borne macrophages from resident brain microglia. What is not known is to what extent macrophages traffic across the BBB following chronic biomaterial implantation. We observed that the spatial area of activated microglia/macrophage immunoreactivity adjacent to implanted materials is the greatest 2 weeks following implantation, and then is observed to be reduced at 4 weeks, and stabilizes thereafter. What is not clear is whether this represents a steady-state reaction, in which the number of activated macrophages trafficking to the implant is equal to the number of macrophages returning to the vasculature, or whether the activated macrophages that arrive from the periphery during acute inflammation remain at the interface for the lifetime of the implant. Defining macrophage kinetics at device surfaces may be clinically relevant, since reducing the number of activated macrophages at the device surface may serve to reduce inflammation, and improve device functionality as well as changes to neurogenesis. Nimmerjahn et al. [107] used EGFP labeling of microglia and a thinned skull preparation to investigate microglial dynamics with *in vivo* two-photon microscopy. Similar methodology could be used to determine the extent of microglial trafficking in vivo, as well as changes to local vasculature and the intact BBB. Distinguishing the contribution of brain derived activated microglial cells at the surface, and the contribution of blood-derived macrophages could be achieved using peripheral

labeling of macrophages using trypan blue [224], or carbon particles [108], and quantifying their presence at the implant site at various timepoints following injury.

As put forth in the introduction, inflammation has been shown to strongly inhibit adult neurogenesis in both animal [174, 175] and clinical experiments [225], which is believed to be associated with learning and memory, and other cognitive functions. In addition, the implantation of various devices have been shown to be associated with cognitive impairments, including hydrocephalic shunts [176-178], deep-brain stimulators [180-183], and devices implanted on the skull that are nonpenetrating [179]. Additionally, the inflammation associated with surgical procedures, in the absence of device placement, has been shown to be associated with cognitive impairments and an increase in the presence of pro-inflammatory cytokines in the hippocampus [219]. In Chapter 4, we showed a significant reduction in markers associated with hippocampal neurogenesis at 12 weeks following the implantation of planar silicon microelectrode arrays, and hollow-fiber membranes, regardless of implant location. In order to more fully correlate inflammation associated with device implantation and clinical cognitive deficits, specific methods using animal models could be employed. One such method is the novel object recognition test [226, 227], in which the amount of time an animal spends exploring a novel object, compared to a familiar object, gives an index of cognitive function. Following surgical implantation of a model device, cognitive function as assessed using the novel object recognition test could be used as a correlate to reduced markers of neurogenesis and increased immunoreactivity for markers of inflammation, thus forming a more clear connection between inflammation, neurogenesis, and cognitive function.

As detailed in Chapter 4, the planar lattice SiMEA showed a reduced inflammatory footprint following 12 week implantation, and was the only implant which did not significantly reduce the number of new DcX<sup>+</sup> neurons as compared to unimplanted controls housed in the same conditions. This indicates that the amount of inflammation and subsequent changes to neurogenesis may be dependent on the amount of surface area implanted. Limited work with a small cohort of animals indicates that reducing the diameter, and hence surface area, can reduce GFAP immunoreactivity surrounding implanted microwires [228], as well as polymeric fibers [229]. Our group has shown that 8 weeks following implantation, a reduction in microglia/macrophage activation is observed at the interface of lattice SiMEAs with the same outer surface as planar SiMEAs, but with approximately 50% less surface area. Little difference in GFAP immunoreactivity was found between the two cohorts, with little to no reduction in NeuN density in the lattice cohort, suggesting that implant size or surface area can significantly change the tissue response [230].

In summary, the field has proven the feasibility, using metal and silicon-based electrodes, to chronically interact with CNS tissue, including signal extraction to control external devices. However, there is still instability associated with such chronic recordings, which is believed to be associated with the biological response to such implants. Previous studies of the tissue response to implanted electrodes in the brain has generally included statistically low-powered studies, and an excessive focus on the astrocyte as the only participant of the tissue response that may affect recording. The field should instead focus on the quantitative tissue response to implanted arrays, the correlation of recording ability to tissue reaction, including the contribution of microglia,

as well as changes to neurogenesis. Such studies would elucidate design inputs to create more biocompatible electrode arrays.

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